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THE UNIVERSITY OF ALBERTA

SENSORY AND CHEMICAL ANALYSES OF FISH TAINTING BY OIL SANDS WASTEWATERS

by

(C)

C. WENDELL KONING

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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OF MASTER OF SCIENCE

IN

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DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA

FALL, 1987

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled SENSORY AND CHEMICAL ANALYSIS OF FISH TAINTING BY OIL SANDS WASTEWATERS submitted by C. WENDELL KONING in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ENVIRONMENTAL SCIENCE.

.....
(Supervisor)
Daniel W. Smith
W. C. Mackay
Lena Kneteman

Date: Oct 7/87

Dedicated to my family;

to my father, Englebert C. Koning

to my mother, Gertrude (1920 - 1964), and to

my mother, Jetske, who became a part of our family in 1973;

to my brothers and sisters -

Bert

Tako

Pauline

Ginny

Phil

Liz

Rita.

ABSTRACT

Concern about fish tainting (off-flavors in fish) has been raised because of the possibility that wastewaters from the oil sands extraction process will be discharged into the Athabasca River in northeastern Alberta.

In order to investigate the potential for fish tainting, rainbow trout were exposed for 24 hours to four different tailings pond derived wastewaters at the Syncrude Canada Ltd. plant, located north of Fort McMurray, Alberta. After 24 hours the fish were sacrificed and filleted. Bile was drawn from the gall bladder. Both fillet and bile samples were immediately frozen and stored for later analysis.

Twenty-five people were screened for their ability to taste and smell various substances. Ten of the best applicants were chosen for the sensory panel. Sensory analysis revealed that all four tailings pond derived wastewaters significantly tainted fish.

Fish-exposure waters, fish bile and fish fillet tissue were chemically analyzed for petroleum compounds. The exposure waters were solvent extracted under base and acid conditions.

Raw bile was analyzed for polyaromatic hydrocarbons by high pressure liquid chromatography with

fluorescence detection. Other bile samples underwent enzymatic hydrolysis in order to analyze for metabolites of petroleum compounds.

Fish fillets were homogenized and solvent extracted. The extracts were passed through gel permeation and florisil columns in order to separate petroleum compounds from fish lipids.

Analysis of the tailings pond waters revealed the presence of alkylated benzenes, alkylated phenols and organic sulphur compounds such as benzothiophene and dibenzothiophene. Analysis of the most-tainted fish revealed the presence of phenol, cresols and dimethyl phenols in both the hydrolyzed bile and tissue. The total concentration of dimethyl phenols in the tissue was well above reported sensory threshold levels. However, because of the presence of other petroleum compounds in the wastewaters and fish tissue, it is unlikely that the phenols were solely responsible for the very strong taint that was detected.

A correspondence was found between concentrations of organic sulphur compounds in the exposure waters and the detectability of taint in the fish tissue.

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TABLE OF CONTENTS

page

ABSTRACT.....v

ACKNOWLEDGEMENTS.....vii

LIST OF TABLES.....xi

LIST OF FIGURES.....xiii

LIST OF ABBREVIATIONS xvi

1. INTRODUCTION 1

2. SCOPE 4

2.1 Problem Statement 4

2.2 Terms of Reference 6

2.2.1 Tailings Pond Wastewater types 6

2.2.2 Fish and Exposure-waters Evaluation 6

2.3 Tailings Pond Description.....8

3. REVIEW OF RECENT RESEARCH 10

3. Reports of Past Oil Tainting Incidents 10

3.2 Tainting Compounds in Tailings Pond Wastewaters 20

3.3 Uptake of Petroleum Compounds by Fish 23

3.4 Northern Considerations 25

3.4.1 Seasonal Effects 25

3.4.2 Natural Erosion of Bitumen Outcrops 26

3.5 Monitoring Methods for Fish Tainting 27

3.5.1 Direct Monitors 27

3.5.2 Indirect Monitors 28

page

4. MATERIALS AND METHODS 31
4.1 Fish Exposure 31
4.2 Sensory Panel Recruitment and Screening..... 32
4.2.1 Screen for Taste Recognition 33
4.2.2 Screen for Odor Recognition 33
4.2.3 Screen for Odor Sensitivity 33
4.3 Sensory Evaluation of Exposed Fish 38
4.4 Water and Wastewater Analysis 40
4.4.1 Base-neutral Solvent Extraction 40
4.4.2 Acid Extraction 41
4.5 Bile Analysis 42
4.5.1 Direct Injection to Liquid Chromatograph 42
4.5.2 Enzymic Hydrolysis of Bile Metabolites 43
4.6 Tissue Processing 44
4.6.1 Blending, and Soxhlet Extraction 44
4.6.2 GPC Separation 45
4.6.3 Florisil CIeanup 47
4.7 Quality Control - Recovery Efficiency 49
4.8 GC Instruments and Conditions 50
4.9 Chemicals 52
5. RESULTS AND DISCUSSION 54
5.1 Sensory Evaluation of Exposed Fish 54
5.2 Water and Wastewater Analysis 59
5.2.1 Gross Physical and Chemical Parameters 59
5.2.2 Alkylated phenols 58

page

5.2.3 Alkylated benzenes 65

5.2.4 Organic Sulphur 71

5.3 Bile Analysis 82

5.3.1 HPLC Results 82

5.3.2 Enzymic Hydrolysis of Metabolites 84

5.4 Tissue Analysis 92

6. CONCLUSIONS 103

7. RECOMMENDATIONS 106

REFERENCES CITED 107

APPENDICES 116

LIST OF TABLES

Page

Table 1. Sample of compounds reported to taint fish which are present in petroleum industry effluents.....19

Table 2. Water solubility of selected petroleum compounds.....21

Table 3. Sensory analysis results of fish exposed to oil sands extraction tailings pond wastewaters, using the "paired comparison" test.....55

Table 4. Temperature, conductivity and pH of the fish exposure waters..... 60

Table 5. Alkylated phenols (µg/L) in treated tailings pond water..... 63

Table 6. Percent recovery of alkylated phenols which had been added to a water sample, solvent extracted under base-neutral and acid conditions.....64

Table 7. GC-FID retention times of phenols in an alkylated phenol standard..... 68

Table 8. Percent recovery of alkylated benzenes which had been added to a water sample, solvent extracted under base-neutral and acid conditions..... 72

Table 9. Alkylated benzenes (µg/L) in treated tailings pond (TTP) water, solvent extracted under base-neutral conditions.....73

Table 10. Organic sulphur in fish exposure waters (solvent extracted under base-neutral conditions only) as detected by GC-FPD, expressed in GC peak areas..... 79

Table 11. Organic sulphur compounds (µg/L) in fish exposure waters (solvent extracted under base-neutral conditions only), as detected by GC-FPD..... 80

Table 12. Alkylated phenols (µg/g) in bile from fish exposed to treated tailings pond (TTP) water; analysis in triplicate.....86

page

Table 13. Percent recovery of selected aromatic compounds which had been added to fish fillet tissue, florisil - hexane fraction.....93

Table 14. Percent recovery of alkylated phenols which had been added onto deactivated florisil columns..... 95

Table 15. Alkylated phenols (µg/kg) in fillet tissue from fish exposed to treated tailings pond water, florisil - DCM fraction 97

Table 16. Data from the sensory analysis performed on fish exposed to tailings pond waters..... Appendix I

Table 17. Statistical table used to determine whether a significant difference in taint occurred between fish exposed to test waters, and fish exposed to Mildred Lake water only..... Appendix I

Table 18. Alkylated phenols (µg/g) in three samples of bile from fish exposed to treated tailings pond water..... Appendix I

LIST OF FIGURES

page

Figure 1. Location of Suncor and Syncrude oil sand extraction plants north of Fort McMurray, Alberta, showing greater detail for Syncrude - on Lease 17 (MacKinnon 1986) 2

Figure 2. Cross section of Syncrude's tailings pond, 1985 (MacKinnon and Boerger 1986) 9

Figure 3. Recording sheet used in screening for the four basic tastes; sweet, salty, bitter, and sour 34

Figure 4. Recording sheet used in the screening for common household odors 35

Figure 5. Recording sheet used in screening for sensitivity towards fish to which varying amounts of naphthalene had been added 37

Figure 6. Scoresheet used for sensory evaluation of fish 39

Figure 7. Percent of sensory panel responses choosing either fish exposed to a test water, or to Mildred Lake water (only) as being more tainted; plus the level of detectability of the evaluated taint 56

Figure 8. Comparison of two GC-FID chromatograms of acid extracts from treated tailings pond water; top - from this project, at 10% of full strength; the bottom - from MacKinnon (1984) 66

Figure 9. GC-FID chromatogram of an alkylated phenol standard 67

Figure 10. GC-MS selective ion chromatograms. Major ion fragments of cresols (peaks 1,2) and dimethylphenols (peaks 3,4,5,6) in treated tailings pond water acid extract (Box A), and in a cresol and dimethyl phenol standard (Box B). Major ion fragment of phenol (94.00 amu) in treated tailings pond water acid extract (Box C), and in phenol standard (Box D) 69

page

Figure 11. GC-MS results. Box B provides a total ion chromatogram of the first 12 minutes of a treated tailings pond water acid extract injection. A mass spectrum of the peak at time 8.133 min. reveals the presence of cresol (Box A), and dimethylphenol at time 10.374 minutes in Box C..... 70

Figure 12. GC-FID chromatogram of a treated tailings pond water base-neutral extract (10% of full strength)..... 74

Figure 13. GC-FID chromatogram of an alkylated benzene standard..... 75

Figure 14. GC-MS TIC of the first 14 minutes of the treated tailings pond water base-neutral extract, (A). Selective ion chromatograms of the major mass fragments of toluene (ion 91) at about 2.5 min., and xylenes (ion 91 and 106) between 4 and 6 min., (B)..... 76

Figure 15. Mass spectra of two peaks found in treated tailings pond water base-neutral extract, revealing the presence of xylenes..... 77

Figure 16. Chromatograms from GC-FPD, for detecting organic sulfur compounds - of base-neutral extracts: A. Mildred Lake water, B. Six percent whole tailings pond water, C. Catchment basin water, D. One-year stored tailings pond water, E. Treated tailings pond water, F. Whole tailings pond water..... 78

Figure 17. HPLC chromatograms of raw bile from: control-trout sampled at (oilsand-free) Winnipeg, Manitoba (upper); trout exposed to treated tailings pond water (middle); and trout exposed to Mildred Lake water only (lower)..... 83

Figure 18. GC-FID chromatograms of 1-naphthol standard (upper); and fish bile to which 1-naphthyl glucuronide had been added, and then enzymatically hydrolyzed (lower)..... 85

Figure 19. GC-FID chromatograms of hydrolyzed bile from trout held in Mildred Lake water only (upper), and in treated tailings pond water (lower)... 87

page

Figure 20. GC-MS comparison of alkylated phenol peaks in a phenol standard (upper box), with those identified in hydrolized bile from fish exposed to treated tailings pond water (lower box).. 89

Figure 21. GC-FID chromatogram of bile extract from fish exposed to 6% whole tailings pond water..... 90

Figure 22. GC-FID chromatograms of bile from trout exposed to treated tailings pond water, revealing the zero effect of buffer (only) on bile hydrolysis (upper), and buffer plus enzyme (lower)..... 91

Figure 23. Xylenes, as revealed by GC-MS, in fillet tissue from fish exposed to treated tailings pond water, florisil - hexane fraction; possibly due only to contaminated hexane..... 96

Figure 24. GC-MS TIC comparison between phenols in a standard (upper box); and phenols identified in fillet tissue of fish exposed to treated tailings pond water, florisil - DCM fraction (lower box)..... 98

Figure 25. GC-MS selective ion detection of dimethyl phenols in fillet tissue of fish exposed to treated tailings pond water, florisil - DCM fraction..... 99

Figure 26. GC-FID chromatograms revealing akylated phenols in fillet tissue of fish exposed to treated tailings pond water (lower), and the absence of phenols in fillet from fish held in dechlorinated water at the University of Alberta (upper), florisil - DCM fraction....100

Figure 27. Comparison between abundance of ions 107 and 122 in fillet tissue of fish exposed to treated tailings pond water(lower box), and those exposed only to Mildred Lake water (upper box), florisil - DCM fraction..... 102

LIST OF ABBREVIATIONS

CB	Catchment basin water, a combination of tailings pond dyke-drainage water and precipitation run-off.
DCM	Dichloromethane
FID	Flame ionization detection
FPD	Flame photometric detection
GC	Gas chromatography
GPC	Gel permeation chromatography
HPLC	High pressure liquid chromatography
I.D.	Internal diameter
KD	Kuderna-Danish (apparatus)
MLK	Mildred Lake water
MS	Mass spectrometry
PAH	Polynuclear aromatic hydrocarbon
PASH	Polynuclear aromatic sulphur heterocycles
TAP	Dechlorinated tap water at the Syncrude Canada Ltd. plant, located north of Fort McMurray, AB.
TIC	Total ion chromatogram
TTP	Treated tailings pond water, which has undergone physical/chemical treatment in order to reduce its toxicity.
UA	University of Alberta dechlorinated tap water.
1YR	Tailings pond water which has been stored for one year in order to reduce its toxicity.
6%	Fresh tailings pond water diluted to six percent of full strength.

1. INTRODUCTION

Approximately 90% of Canada's oil supply is contained in heavy oil deposits, the majority of which are located in the oil sands deposits of Alberta. Oil sands are characterized by unconsolidated clays and sands saturated with highly viscous hydrocarbons. The Alberta oil sands, located in four major deposits, are estimated to contain 150×10^9 m^3 of heavy oil or bitumen.

The Athabasca oil sand deposit is the largest in Alberta, covering an area of approximately 31,000 km^2, north of Fort McMurray. Currently this deposit is being surface mined, and the bitumen extracted and upgraded to synthetic crude by Syncrude Canada Ltd. and Suncor Inc. Figure 1 provides a map depicting the location of the two plants, giving greater detail for the Syncrude operation.

According to Boerger and Aleksiuk (1987), Syncrude produces over 6.4×10^6 m^3 of synthetic crude annually from this deposit, which results in an annual production of 100×10^6 m^3 of toxic wastewater which is stored in a tailings pond. Currently 70% of this wastewater is recycled, resulting in a net annual accumulation of $20\text{-}30 \times 10^6$ m^3 wastewater in the tailings pond. The tailings pond may eventually hold 450×10^6 m^3 of wastewater with a final depth of 70 meters (MacKinnon and Boerger 1986).

NOTE:

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The material that was removed contained a map showing the location of the Suncor Inc. and Syncrude Canada Ltd. oil sand extraction plants located north of Fort McMurray, Alberta.

The original source of this material is:

MacKinnon, M.D. 1986. Environmental Aspects of waste water management at an oil sands development in northern Alberta. In: Proceedings of S.P.I.B. Conference on "Northern Hydrocarbon Development, Environmental Problem Solving", Sept. 24-26, 1986. Banff, Alberta. 18 pp.

Eventually the entire mining site including the tailings pond must be decommissioned and decontaminated. One option under consideration is discharge of the tailings pond water into the nearby Athabasca River. Concerns have been raised in this respect about the need to protect the Athabasca River fishery, in particular the potential for fish tainting (off-flavors in fish) as a result of tailings pond contaminants.

2. SCOPE

2.1 PROBLEM STATEMENT

In the production of synthetic crude oil from oil sands, bitumen is extracted from the sand grain matrix by the addition of caustic—hot water and steam in a conditioning drum. From here the mixture is discharged into separation cells where the sand grains separate by gravity to produce a bottom tailings stream. Additional bitumen is extracted from the remaining suspended clay fines and water mixture by air flotation. This bitumen, plus the initially recovered bitumen froth is diluted with raw naphtha and then centrifuged, producing another water-sludge effluent.

This bitumen extraction process, called the "Clark hot water process" requires approximately 1.5 m^3 of water per tonne of oil sand feed. The resulting wastewater is a slurry of water, solids and non-extracted bitumen, 50:50:1 by weight (MacKinnon 1986). In addition there is some contamination from the diluent naptha.

Bitumen extracted from the oil sand is unsuitable for pipeline shipment because of its high viscosity and high sulphur content. Therefore, on-site upgrading facilities crack the high molecular weight bitumen molecules by coking processes to produce lighter

fractions, which are blended to produce a high quality synthetic crude oil. The water uses and resulting wastewaters from bitumen upgrading are similar to those of a petroleum refinery, namely, process condensates, boiler blowdown, cooling tower blowdown and storm drainage (Hrudey and Scott 1981).

Suncor (formerly called Great Canadian Oil Sands - GCOS) was the first plant in operation. Their operating licence allows for the discharge of upgrading plant wastewaters after certain treatment procedures.

At present Syncrude follows a "zero discharge" policy, in which no process waters are discharged to the Athabasca River. All oil sand extraction and upgrading effluents are stored in the tailings pond. According to their operating permit, however, Syncrude is required to conduct research into the reclamation of the tailings pond area so that it can be reclaimed as a viable water body after abandonment, or safely discharged into surrounding surface waters (Mackinnon and Retallack 1981).

As a future tailings pond management option, Syncrude is considering discharging certain types of tailings pond wastewaters. Apart from problems of acute toxicity to aquatic organisms, which have been previously researched (Boerger and Aleksiuk 1987), questions have been raised concerning the potential for fish tainting

from these tailings pond wastewaters. Currently there are no published studies evaluating the fish tainting potential of tailings pond wastewaters.

2.2 TERMS OF REFERENCE

2.2.1 Tailings Pond Wastewater Types. To evaluate the tainting potential of tailings pond wastewaters, rainbow trout (*Salmo gairdneri*) were exposed to one of the following:

1. Six percent fresh whole tailings pond water (6%). Since tailings pond water is acutely toxic at 7 - 10% (MacKinnon and Boerger 1986), whole tailings pond water was diluted to a final strength of 6% by the addition of Syncrude's dechlorinated tap water.
2. One year stored tailings pond water (1YR). According to MacKinnon and Boerger (1986) natural detoxification of surface tailings pond water occurs over a period of 12 months when left without any additional wastewater input in shallow, well aerated pits. The result is a non-acutely toxic water. Fish were expected to survive in full strength one year stored tailings water for the duration of a 96 hour LC_{50} bioassay test.
3. Treated tailings pond water (TTP). According to

MacKinnon and Boerger (1986), tailings pond water can be detoxified rapidly by chemical treatment which involves coagulation at a pH between 4.5 - 5.0, followed by flocculation with an anionic polyelectrolyte. Using this method, Makinnon and Boerger (1986) report 100% survival of rainbow trout exposed to full strength treated tailings pond water during a 96 hour static bioassay.

4. Catchment basin water (CB). The tailings pond dyke is made of sand which allows for some outward seepage to occur. Dyke-drainage waters are collected in ditches on the perimeter of the tailings pond, which then drain into a catchment basin. Together with inputs of precipitation and run-off, this material is pumped back into the tailings pond.

The four tailings pond waters that were used in this project do not pose acute toxicity risks for aquatic organisms in the Athabasca River. However none of them had been tested for their ability to taint fish.

2.2.2 Fish and Exposure-waters Evaluation. Sensory evaluation, the main focus of this project, was performed on all fish which had been exposed to tailings pond waters. Exposed fish having a detectable taint had their tissue and bile analyzed for those petroleum compounds

that have been reported to potentially taint fish. Fish exposure waters were analyzed for base-neutral and acid extractable compounds.

2.3 TAILINGS POND DESCRIPTION

Syncrude began operations in mid-1978 and expects to have an operational lifespan of 25 years, ultimately producing over one billion barrels (1.6×10^8 m^3) of oil. In the process, as part of their "zero discharge policy", the tailings pond will continue to grow.

By the end of 1985 the water surface of the tailings pond covered an area of approximately 14 km^2, had depths approaching 40 meters, and had a pond volume of 2.0×10^8 m^3 (MacKinnon and Boerger 1986).

The pond consists of an upper zone of free water which has a concentration of solids less than 1%. The sludge zone, located below 10 meters depth, contains up to 45% solids which result from settling and consolidation of the fines added to the pond. The tailings pond profile is illustrated in Figure 2.

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The material that was removed contained an illustration showing a profile of the tailings pond at Syncrude Canada Ltd.

The original source of this material is:

MacKinnon, M.D. and H. Boerger. 1986. Description of two treatment methods for detoxifying oil sands tailings pond water. Water Pollution Research Journal of Canada. 21(4)496-512.

3. REVIEW OF RECENT RESEARCH

3.1 REPORTS OF PAST OIL TAINTING INCIDENTS

Fish tainting incidents caused by petroleum compounds have been reported since the world-wide establishment of oil refineries and petrochemical industries with their corresponding wastewater discharges into freshwater and marine systems.

In the early 1950's an oily flavor was reported in fish caught in the Bow River downstream from Calgary, Alberta. In a study by the Alberta Department of Health (Anon 1958), rainbow trout were exposed to various dilutions of effluent from a local refinery. When exposed for 24-48 hours to 1% and 5% concentrations of API separator effluent, fish were found to have an oily flavor. API separator effluent contains the water-soluble fraction of oil. In a follow-up study, Krishnaswami and Kupchanko (1969) exposed rainbow trout to a Calgary refinery API separator effluent for 6 - 24 hours, which resulted in a detectable taint as determined by a five member sensory panel.

In the spring of 1972, fish caught under the ice in the Athabasca River downstream from Jasper, Alberta were judged unfit to eat by sport fishermen, which prompted Ackman and Noble (1973) to analyze the fish

(mainly whitefish - species not reported) for the presence of tainting compounds. Steam distillation of fish tissue revealed the presence of hydrocarbons (n-alkanes) in the tainted fish similar in appearence to those from a sample of diesel oil. Chromatograms of extracts from untainted control fish caught in Lake Minnewanka did not reveal a similar pattern. Diesel oil entering the Athabasca River through a discharge pipe located below the Canadian National Railway yards in Jasper was suspected of being responsible for the detected taint.

During the winter of 1981-1982 a series of accidents took place at the Suncor Inc. oil sands plant in northeastern Alberta whereby substantial volumes of petroleum compounds were discharged into the Athabasca River. Subsistence and commercial fishermen as far away as Lake Athabasca, 300 km downstream from the discharge point complained about walleye and whitefish having an odor and taste similar to kerosene. The 1982 commercial fishery was forced to close. Experimental tainting studies at the Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba confirmed that the material discharged by Suncor could taint fish after only a few hours exposure (Hamilton et al. 1987). Suncor was eventually charged and convicted under the Fisheries

Act on three counts of releasing a substance deleterious to a fishery (Hrudey and Nelson 1986).

In Michigan, Fetterolf (1964) responded to complaints of fish tainting (in particular tainted walleye) occurring under ice during winter. He obtained untainted rainbow trout from a local fish hatchery and transported them to the complaint site by truck. Cages were suspended 0.6 metres under the lake ice in a location where the fish would be exposed to the refinery effluent plume. The fish were exposed for two weeks. Tainting was confirmed by five trained judges using a rating scale from 1 - 7, based on fish flavor and aroma. No statistical comparison of the sensory results, nor any analysis of petroleum compounds in lake water or fish tissue were performed. However, a fluorescent dye released in the refinery effluent on two occasions was found both times in the complaint area two days later.

After a diesel oilspill of more than 2000 litres in a Northern Ireland river, area fishermen complained that the fish were inedible due to a strong diesel fuel taint. Mackie et al. (1972) analyzed brown trout (*Salmo trutta* L.) caught nine days after the spill. Samples of exposed trout were cooked for sensory analysis and compared to control trout. The trout flesh suspected of being tainted had an aroma and taste strongly reminiscent

of diesel fuel oil, whereas the control trout did not. Additional fish were eviscerated, minced and solvent extracted for chemical analysis. The resultant extract was fractionated into petroleum compounds and fish lipid by repeated elution through a silicic acid column. GC, GC-MS (refer to the List of Abbreviations) revealed the presence of hydrocarbons (n-alkanes) in tainted fish similar to those in diesel fuel. These were not present in untainted fish. No aromatic hydrocarbons were identified by this method. Neither n-alkane concentrations, nor percent recovery were determined.

Australian mullet were found on occasion to smell like kerosene by Vale et al. (1970). Further work by Shipton et al. (1970) found that volatile compounds extracted from the tainted fish by vacuum sublimation were very similar, both qualitatively and quantitatively to those in a commercial kerosene sample when analyzed by GC, GC-MS. They identified mainly alkanes with a small quantity of aromatics.

The results of Shipton et al. (1970) were confirmed by Connell (1974). Mullet were caught, cleaned, wrapped in aluminum foil and stored at below 0 ^{o}C prior to examination. Sensory analysis was performed by placing fish fillets in boiling water and assessing whether a taint was detectable in the produced vapors. Those fillets

identified as tainted were subjected to steam distillation with the resultant volatile fraction analyzed by GC, GC-MS. Connell thereby identified n-alkanes in the tainted fillets similar to those found in kerosene.

In Japan, as early as the 1950's, marine fish caught near coastal petroleum-related industries were reported to have obnoxious odors (Ogata and Miyake, 1973). Deshimaru (1971, cited by Ogata and Miyake 1975) reported an oily odor in fish after they were exposed to oil added to flowing water.

Ogata and Miyake (1973) identified toluene as a compound with tainting potential. They exposed eels to seawater containing toluene and found they had the same odor as eels caught near oil refineries located on the Japanese coast.

Ogata and Miyake (1975) exposed eels for 1, 3, and 7 days to Arabian Light Crude diluted to 800 ppm with freshwater. The eels were prepared for sensory analysis by peeling their skin off, and cutting their flesh in slices. The slices were cooked in boiling water with salt for a few minutes, and then presented to a sensory panel. In blind tests, six panelists found that the eels tasted bitter and had an oily smell. Eels exposed to crude oil for 7 days had a stronger oil-like odor and bitter taste than those exposed for 1 and 3 days. Volatile organic

compounds were purged from the eel flesh by a stream of inert gas. Analysis of the volatile compounds occupying the head-space above the purged tissue revealed the presence of benzene, toluene and o, m, and p-xylene in the tainted eels, the concentrations of which increased with exposure time. These compounds were not detected, or barely detected in control eels raised in clean water.

Ogata et al. (1977) exposed eels to 1000 ppm Arabian Light Crude. Sensory analysis was performed as in Ogata and Miyake (1975). All judges detected obnoxious odors in flesh from the exposed eels. Eel tissue samples were saponified, fractionated, and then analyzed by gas and thin layer chromatography. Results revealed the presence of paraffins (alkanes), as well as benzene, toluene, and o, m, and p-xylene. Analysis by GC-FPD revealed the presence of organic sulphur compounds.

Further research was performed by Ogata and Miyake (1978, 1980), and Ogata and Fujisawa (1983, 1985). Eels, oysters and mussels were exposed to petroleum samples, then sacrificed, their flesh homogenized and saponified. Petroleum compounds were separated from lipid material using silica gel alumina column chromatography. These extracts, analyzed by GC-FID/FPD, and GC-MS confirmed the transferability of potential tainting petroleum compounds such as alkylated benzothiophenes and

dibenzothiophenes, and alkylated benzenes to marine organisms, including marine fish.

The work of Ogata and Miyake (1975, 1978, 1980), Ogata et al. (1977), and Ogata and Fujisawa (1983, 1985) is supported by Jardine and Hrudey (1987) who performed sensory evaluation on walleye muscle tissue to which test compounds had been added, and found detection thresholds of 9 mg/kg for p-xylene; 4.67 mg/kg for dibenzothiophene; and 0.12 and 0.09 mg/kg for benzothiophene.

Naish and Brouzes (1980) exposed rainbow trout to various pulp and paper effluents. They subsequently digested the fish tissue at high pH and extracted organic compounds with hexane or ether, followed by florisil separation-cleanup with ether eluant. For wastewater characterization they used acid extraction into diethyl ether and florisil separation. A well trained sensory panel was employed with proper sampling facilities. They found the process stream which contained both totally reduced sulphur compounds and phenol from the recovery flue gas caused the greatest taint. Even though the fish for tissue extraction were placed in the maximum sublethal concentration of the effluent, they could not find the compounds in the tissue. Either the method used was insensitive to isolating the tainting compounds, or the compounds responsible for the taint do so at a very low

concentration. Their experience certainly illustrates that fish tainting may occur at levels below the analytical limit of most instruments.

Phenol, in concentrations ranging from 10 to 4,500 ppb, has been identified as the predominant priority pollutant in the acid-fraction of Canadian petroleum industry effluents (PACE 1981, cited by Hargesheimer et al. 1984). Winston (1959, cited by Fetterolf 1964), found fish were tainted when exposed to 1 mg/L phenol.

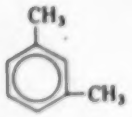
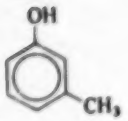
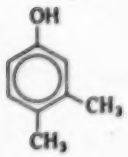
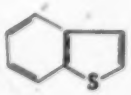
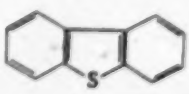
Shumway and Palensky (1973) report that rainbow trout were tainted after 48 hours exposure to 0.2 mg/L and 0.12 mg/L m- and p-cresol, respectively, and after 96 hours exposure to 0.45 mg/L o-cresol. Bandt (1955, cited by Persson 1984) reports that exposure to 1 mg/L of 2,4 and 2,5 and 3,5 dimethyl phenol created a taint in roach (*Rutilus rutilus* L.), and exposure to 5 mg/L 3,4 dimethyl phenol tainted carp (*Cyprinus carpio* L). Durations of the dimethyl phenol exposures were not reported.

Much of the reported literature on phenol-induced tainting occurred before any widespread use of reliable analytical techniques for trace organics detection. Some of the threshold levels reported are therefore of questionable accuracy. However the potential for phenols to taint fish is notable and begs further testing.

Jardine and Hrudey (1987) report a detection threshold of 0.21 mg/kg for 2,5 dimethyl phenol which had been added to walleye flesh. This threshold is substantially lower than the thresholds they reported for p-xylene and dibenzothiophene, and comparable to the threshold they reported for benzothiophene.

Table 1 provides structural formulae of compounds from petroleum industry effluents which were identified as potential tainting compounds in the investigations reviewed in this section, and in fish tainting reviews by Connell and Miller (1981) and Motohiro (1983).

Table 1. Sample of compounds reported to taint fish which are present in petroleum industry effluents.

toluene	
xylenes - example: 3-xylene	
phenol	
cresols - example: 3-cresol	
dimethyl phenols - example:3,4-dimethyl phenol	
benzothiophene - and alkylated derivatives	
dibenzothiophene - and alkylated derivatives	

3.2 TAINTING COMPOUNDS IN TAILINGS POND WASTEWATERS

Birkholz et al. (1987) analyzed the water-soluble fraction of a wastewater oil sample from the Suncor oil sands plant. This wastewater discharge draws primarily from the bitumen upgrading portion of the plant. They found aromatic compounds to be the predominant group of petroleum compounds in this water-soluble fraction. Table 2 provides a comparison of the water solubility of various petroleum compounds. According to Birkholz et al. (1987) the water-soluble fraction of oil is of particular concern because it can achieve intimate contact with fish and other pelagic organisms, and this fraction is not removed by gravity oil separation devices. In this fraction they identified and absolutely confirmed the presence of alkylated benzenes including o, and m-xylene, alkylated benzothiophenes and dibenzothiophenes.

Availibility of the water-soluble fraction of oil to fish is supported by Murray et al. (1984). Using "purge and trap" head space analysis, Murray et al. determined that fish exposed to 100 ppm Norman Wells crude contained the same compounds in their tissue as in the water-soluble fraction of the crude oil.

Ritchie et al. (1979) pyrolyized Athabasca bitumen asphaltene at 350, 500, and 800 ^{o}C, and performed GC-MS analysis on the condensible volatiles. He identified

Table 2. Water solubility of selected petroleum compounds.

COMPOUND	WATER SOLUBILITY (mg/L) at	WATER TEMPERATURE (°C)	REFERENCE
n-pentane	47.6	25	Polak and Lu (1973)
2,4-dimethyl pentane	5.5	25	Polak and Lu (1973)
n-hexane	12.4	25	Polak and Lu (1973)
2,2,5-trimethyl hexane	0.54	25	Polak and Lu (1973)
n-octane	0.85	25	Polak and Lu (1973)
benzene	1,755.	25	Polak and Lu (1973)
toluene	573.	25	Polak and Lu (1973)
4-xylene	185.	25	Polak and Lu (1973)
naphthalene	30.	NP[a]	Vassilaros et al. (1982)
anthracene	0.04	NP	Vassilaros et al. (1982)
benz[a]-anthracene	0.01	NP	Vassilaros et al. (1982)
benzo-thiophene	113.	NP	Vassilaros et al. (1982)
dibenzo-thiophene	1.7	NP	Vassilaros et al. (1982)
Phenol	82,000.	15	Verschueren (1983)
4-cresol	24,000.	40	Verschueren (1983)
2,4 dimethyl phenol	7,867.	25	Banerjee et al. (1980)

aNP - Temperature at which solubility measured was not provided.

a number of aromatics including alkylated benzothiophenes, alkylated dibenzothiophenes, and the most abundant - alkylated benzenes. The procedure used by Ritchie et al. (1969) is not specifically used at Syncrude or Suncor, (1986), however both plants do use high temperature coking to convert bitumen to synthetic crude. Strosher and Peake (1976) conducted a detailed survey of Great Canadian Oil Sands (GCOS - now Suncor) wastewaters. They found 3.0 - 3.8 mg/L (complex) phenols in the tailings pond dyke drainage, and 0.18 mg/L (simple) phenols in the upgrading plant wastewater. Strosher and Peake (1978) found 0.11 - 0.19 mg/L phenols in Suncor upgrading plant wastewaters.

Hrudey et al (1976) report 0.030 mg/L (simple) phenols in GCOS (Suncor) tailings pond dyke drainage water and 0.012 mg/L phenols in the bitumen upgrading plant effluent.

Hargesheimer et al. (1984) identified 31.0 - 152.0 ppb (µg/L) phenol, 25.0 - 122.0 ppb cresols and dimethyl phenols (no concentration given) in Syncrude tailings pond waters sampled between 2 - 15 metre depths. As well they measured 2.0 ppb phenol and less than 1 ppb cresols in tailings pond dyke drainage. The catchment basin contained less than 1 ppb phenol and cresols.

Mackinnon and Boerger (1986) analyzed tailings pond water and report 0.15 ppm (mg/L) phenols in fresh

tailing pond water; 0.01 ppm phenols in tailings pond water after one year of storage; the same after two years storage; and 0.13 ppm phenols in tailings pond water after chemical treatment by acidification followed by flocculation with an anionic polyelectrolyte.

The differences in reported phenol levels likely reflects differences in precision and accuracy of various analytical techniques used.

3.3 UPTAKE OF PETROLEUM COMPOUNDS BY FISH

Uptake of petroleum compounds by fish can occur via gills, epidermis, and the alimentary canal. For marine fish, uptake via the alimentary canal provides an important hydrocarbon contribution since they are required to drink large volumes of seawater for osmoregulation. Most sources consider gill uptake the primary uptake route in freshwater fish (Persson 1984). Their conclusion is based on finding the highest concentration of petroleum compounds in the gills and liver of exposed fish. Uptake of petroleum compounds via the alimentary canal would occur during feeding.

Uptake of petroleum compounds also varies between freshwater fish species, depending on habitat and food source. For example in the Athabasca River, two important commercial fish are walleye (*Stizostedion vitreum*) and lake whitefish (*Coregonus clupeaformis*). Walleye are

predators inhabiting the entire water column, whereas whitefish are bottom feeders. Because of their differences in habitat and food source, one might expect differences in their uptake of petroleum compounds and their subsequent tainting potential.

Connell and Miller (1981) reported that there was a greater storage of aromatic hydrocarbons in lipid-rich than in lipid-poor fish species because of the affinity between hydrocarbon and lipid materials. Lipid-rich lake whitefish and rainbow trout therefore may be more susceptible than leaner fish species such as walleye to petroleum tainting.

The rate of uptake of odor-causing petroleum compounds in fish can be quite rapid, in fact a detectable taint may occur within minutes of exposure (Shumway and Palensky, 1973). The rate of uptake is affected by factors such as compound concentration, location of the petroleum compounds with respect to location in the water column or sediments, exposure duration, water temperature, the fish species and the physiological state of the fish (Persson 1984).

Synergism, in which the effect of two or more substances occurring together is greater than the simple addition of the effects of the substances if they occurred by themselves, may also be a factor in fish tainting.

Rosen et al. (1962) noted enhanced odor resulting from the interaction of organic contaminants such as naphthalene, acetophenone, and phenyl methyl carbinol in water. Mann (1962, cited by Persson 1984) found that uptake of phenols by fish was enhanced in the presence of detergents. Sidhu et al. (1970) found that adding 4 ppm of "Tween-60", a commercial detergent to 7.5 ppm commercial kerosene in seawater resulted in a much stronger fish taint.

3.4 NORTHERN CONSIDERATIONS

3.4.1 Seasonal Effects The unique character of northern waters must be taken into account when considering the potential tainting impact of oil containing effluents.

In the oil sands area, the Athabasca River is covered with ice for six months of the year. During the ice-cover period, volatilization of lower molecular weight, more volatile petroleum compounds is prevented, resulting in extended exposure to fish. As well, the Athabasca river flow is lowest from January to March. The dilution factor for effluent input is therefore reduced during winter which increases the potential for tainting. The Suncor mishap which was linked to fish-tainting (Section 3.1) occurred during winter.

Apart from the warmer summer months, cold water temperatures will likely reduce microbial degradation of petroleum compounds discharged in tailings pond effluents. Also, low nutrient levels in the Athabasca River, in particular nitrogen and phosphorous may be limiting factors affecting microbial degradation of the petroleum contaminants (Costerton and Geesey, 1979).

3.4.2 Natural Erosion of Bitumen Outcrops The Athabasca River downstream of Fort McMurray flows through natural outcrops of bitumen which are subject to erosion. This input of petroleum compounds may provide a measure of tolerance to additional inputs from manmade sources in that it may have already sensitized the microbial population to utilize petroleum effluents as a food source, and induced higher organisms to organize the appropriate enzymatic pathways in order to metabolize and excrete petroleum compounds - rather than accumulate them.

Hamilton et al. (1987), commenting on the comparison of oil sand extraction effluents with bitumen erosion products state that although the types of petroleum compounds in each source are the same, the relative composition is very different, with the lower molecular weight, more volatile, more water soluble, and

often more toxic (and more tainting) aromatic compounds being much more abundant in the oil sand extraction effluent than in the bitumen erosion products. The potential for fish tainting by oil sand extraction effluents is therefore likely far greater than by natural bitumen erosion products.

3.5 MONITORING METHODS FOR FISH TAINTING

3.5.1 Direct Monitoring. The most effective method to monitor fish tainting is by operation of a well-trained, permanent sensory panel operating in proper evaluation facilities. A small highly trained panel will give more reliable results than a large untrained panel (Larmond 1977). A laboratory panel is usually composed of ten to twenty persons with three or four replications by each judge for each treatment, or fish exposure (Larmond 1977).

If odor is being evaluated, analysis ought to be performed under red lighting in order to avoid evaluations biased by fish flesh color (Iredale and York 1976). As well preparation of fish samples ought to be done in a way that minimizes losses of volatiles.

According to EVS (1985) most of the compounds that have been implicated in tainting are volatile low-

molecular weight components detected mainly by odor rather than taste. Kerkhoff (1974, cited by Vandermeulen 1987) notes that few if any petroleum compounds can normally be detected by taste alone. According to Appel (1985) aroma, perceived in the nasal passages contributes about 75-80% of the impression of flavor in food. Sensory evaluation of fish tainting by odor alone should provide most of the response available by taste.

Proper sensory evaluation may be too time consuming and costly to be used as a routine monitoring procedure. Consequently, there is value in establishing what level of contaminant may be expected to taint.

3.4.2. Indirect Monitors. A number of markers have been suggested as indicators of the presence of oil pollution.

Organic sulphur, of petroleum origin, in fish tissue could be used as a marker of oil pollution in fish according to Ogata and Miyake (1980). A correlation with fish tainting might be found. However this would still entail fish capture, tissue extraction, extract cleanup, and the use of sophisticated detectors such as GC, GC-FPD, and GC-MS, which would make it difficult to use as a routine cost and time effective monitoring procedure.

Metabolism of petroleum compounds requires a cytochrome-P450 dependent mixed-function oxidase (MFO) system. The MFO system is a non-specific complex of microsomal enzymes which mediates the metabolism of lipophilic petroleum compounds by rendering them more water soluble and thereby easier to excrete. The MFO system is inducible by fish exposure to petroleum compounds. Attempts have been made to measure this induction and correlate induction with oil pollution levels. However Vandermeulen (1987) researched the kinetics of MFO induction in brook trout and found that MFO activity was indeed enhanced when fish were exposed to various oils but found it difficult to correlate the level of MFO induction to either dosage or length of exposure. A correlation with fish tainting and MFO induction may also prove difficult.

Fish bile can be analyzed by direct injection HPLC with fluorescence detection. Correct selection of wavelengths can be used to monitor for the metabolites of diaromatic and polyaromatic hydrocarbons. A correlation between concentrations of hydrocarbon metabolites in bile and the prevalence of liver lesions was found by Krahn et al. (1984, 1986) and Malins et al. (1985a, 1985b). They, as well as Statham et al. (1976), and Morgan et al. (1987) suggest using fish bile as a relatively simple and

inexpensive monitor of oil pollution in fish. No correlation has yet been made with fish tainting however.

Currently, the only effective method for monitoring potential fish tainting is by sensory evaluation of exposed fish. A less expensive, less time consuming monitor has not been found.

In this investigation an attempt was made to correlate sensory evaluation results with petroleum compound concentrations in the bile and tissue.

4. MATERIALS AND METHODS

4.1 FISH EXPOSURE

Rainbow trout, reared at the provincial government hatchery at Calgary, were transported to the Syncrude site, north of Fort McMurray, by truck. The average fish weight was 61.2 g; average length (fork length) was 172 mm. Upon arrival the fish were transferred to a 1 m^3 nalgene container, which received a continuous flow of water from nearby Mildred Lake.

Fish were exposed to test waters in shallow, 0.5 m^3 polyethylene pools located in a warehouse on the Syncrude site. Fish were exposed to one of the following tailings pond derived waters:

1. Fresh tailings pond water - diluted to 6% of full strength (6%). Dechlorinated tap water at Syncrude was used as the diluent.
2. One year "stored" tailings pond water (1YR) - stored to reduce toxicity.
3. "Treated" tailings pond water (TTP) - treated by acidification/chemical flocculation.
4. Catchment basin water (CB) - a combination of tailings pond dyke-drainage water, and precipitation runoff, which drain together into a common basin.
5. Syncrude dechlorinated tap water (TAP).

From 7 to 11 fish were held in each exposure pool. Aerated conditions were maintained throughout the 24 hour exposure period. Water temperature, pH, and conductivity were measured before and after each exposure.

After 24 hours exposure the fish were sacrificed, cleaned, and gutted. Similar treatment was performed on 46 fish held only in Mildred Lake (MLK) water, and 13 fish held in dechlorinated water at the University of Alberta (UA). All fillets, with skin intact, were weighed and individually vacuum sealed in polyethylene bags. At the same time, bile was drawn from the gall bladder using 1000 µL syringes, and stored in 300 µL polyethylene, ultracentrifuge vials. Both fish fillets and bile samples were immediately frozen, and later stored at -25 ^{o}C, until required for analysis.

4.2 SENSORY PANEL RECRUITMENT AND SCREENING

Twenty-five people were recruited and screened for their sensory ability using a procedure adapted from Vaisey-Genser (1977). Screening consisted of a series of tests to determine each person's ability to recognize a variety of tastes and odors. Ten of the most sensitive recruits were then chosen for the sensory panel.

4.2.1 Screen for Taste Recognition To screen for tasting ability, each recruit was given eight clear unidentified solutions to sample. The recruit identified the taste by indicating whether it was sweet, sour, salty, bitter or tasteless. The sweet, sour, salty and bitter solutions were made by the addition of sucrose, citric acid, salt and quinine, respectively. The tasteless solutions contained only tap water. The recording sheet used is given in Figure 3.

4.2.2 Screen for Odor Recognition To screen for odor recognition, recruits were given unidentified solutions containing the following added odors: cloves, household bleach, almond extract, onion, vanilla extract, soap, ethanol, vinagar, tuna meat, bitumen, iodine and curry. Recruits were asked to identify each odor as closely as possible. The recording sheet used is given in Figure 4.

4.2.3 Screen for Odor Sensitivity The final screening test, to determine sensitivity to an odor, consisted of three separate sessions during which recruits were tested for their ability to discriminate between walleye flesh to which concentrations of 0, 2.0, 4.0, and 8.0 mg/kg naphthalene had been added. Naphthalene, a diaromatic hydrocarbon, has a mothball-like odor.

SCREENING TEST FOR TASTE

NAME_____ DATE_____

Eight coded samples are provided. Each of these cups contains weak water solutions of chemicals representing the four basic taste sensations. One or more of these may be a "blank" of distilled water, or may be a duplicate sample.

Rinse your mouth with the water provided and take a bite of cracker before tasting each sample. Taste each sample separately and in the order indicated. For each sample, record under "Taste Description" if the sample is tasteless, or has a sweet, salty, sour or bitter taste.

Sample Code Number	Taste Description
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Figure 3. Recording sheet used in screening for the four basic tastes, sweet, salty, bitter and sour.

SCREENING TEST FOR ODOUR

NAME_____ DATE_____

Ten coded samples are provided. Each of these test tubes contains a dilute solution of a compound having a typical odour
Sniff each sample separately and in the order indicated. Rinse your mouth with the water provided and take a bite of cracker after smelling each sample. Wait approximately 15 seconds between samples. Record your description of each sample under "Odour Description".

Sample Code Number	Odour Description
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Figure 4. Recording sheet used in screening for common household odors.

For this final screening test, walleye fillets were prepared and presented to the recruits using a procedure adapted from Iredale and York (1976). The walleye fillets were ground up in a Waring blender. Naphthalene dissolved in ethanol was then added (spiked) to the ground up fillets. Regardless of the amount of naphthalene added, each walleye sample received the same volume of ethanol carrier. Prepared walleye were then divided into 5 g lots, wrapped in 200 mm by 200 mm squares of aluminum foil, and vacuum sealed in polyethylene bags. Bagged fish samples were placed in a hot water bath at 40 to 60 ^{o}C in order to maximize their aroma. Recruits were requested to open a bag, tear open the tops of a pair of aluminum packets, hold the open packets to their nose, inhale, compare the two samples, and record which sample contained the greater perceived concentration of naphthalene. Before evaluating the next fish pair, panelists were requested to wait 15 seconds, and to consume a small portion of the unsalted soda biscuits and dilute lemon water provided, to remove lingering taste and odor sensations from the palate. The recording sheet used is given in Figure 5.

All screening activities and subsequent sensory sessions were performed in a specially equipped, sensory evaluation room. Individual booths were provided for each

Sequential Analysis Set No _____

SCREENING TEST FOR FISH TAINTING

NAME_____DATE

Seven pairs of coded samples are provided. Each of these foil packets contains a sample of fish. Some samples have been artificially "tainted" with varying concentrations of a common household substance (moth balls). Other samples are untainted.

To evaluate the samples, tear off the end of the foil packet and open it as much as possible. Sniff each pair of samples separately and in the order indicated. Circle the sample within each pair which is most tainted. After you have completed your evaluation, close each packet by folding over the open end.

Rinse your mouth with the lemon water provided and take a bite of cracker after smelling each sample pair. Wait approximately 15 seconds between sample pairs.

Pair	Samples
1	_____
2	_____
3	_____
4	_____
5	_____
6	_____
7	_____

Figure 5. Recording sheet used in screening for sensitivity towards fish to which varying amounts of naphthalene had been added.

panelist. Each booth was provided with a faucet and sink, hot plate, and waste container. To block any perception of fish discoloration, all sensory evaluation was performed under red lighting.

4.3 SENSORY EVALUATION OF EXPOSED FISH

Fish exposed to Mildred lake water and tailings pond waters were prepared in a similar manner to those in the third screening session, (Section 4.2.3). Fish samples not required immediately were frozen for later use.

Sensory evaluation was performed using a paired comparison test described by Larmond (1977), with statistical tables from Roessler et al. (1956). In this test, a pair of fish samples were compared on the basis of their odor. Panelists were informed that in each pair, one member was the "reference" sample, the other member a "test", i.e., exposed sample. Panelists were required to choose one member as being more tainted. A nine point scale allowed the panelists to score the detectability of taint, i.e., from "barely detectable" to "highly detectable". The score sheet used is provided in Figure 6.

Fish exposed only to Mildred Lake water only were used as the "reference" fish. Each of the 10 panelists compared a test fish sample to a reference three times, for a total of 30 judgements. To determine whether

SCORING OF FISH TAINTING

NAME _____ DATE _____

Seven pairs of coded fish samples are provided. In each pair, one of the samples contains fish that has been exposed only to natural lake water. The other sample contains fish that has been exposed to either to tap water, or to various wastewaters associated with oil sands mining and upgrading operations.

To evaluate the samples, tear off the end of the foil packet and open it as much as possible. Sniff each pair of samples separately, and in the order indicated. Circle the sample within each pair which is most tainted. Evaluate the degree of taint of this sample by ticking within the most appropriate category (see example below).

Rinse your mouth with the lemon water provided and take a bite of cracker after evaluating each sample pair. Wait approximately 15 seconds between sample pairs.

Pair No.	Samples		Detectability of Taint
Example	123	(456)	barely detectable / detectable ✓ / highly detectable
1	622	342	barely detectable / detectable / highly detectable
2	332	519	barely detectable / detectable / highly detectable
3	421	387	barely detectable / detectable / highly detectable
4	820	153	barely detectable / detectable / highly detectable
5	910	321	barely detectable / detectable / highly detectable
6	983	396	barely detectable / detectable / highly detectable
7	492	329	barely detectable / detectable / highly detectable

Figure 6. Scoresheet used for sensory evaluation of fish.

Mildred Lake imparted an off-odor to fish, each panelist compared Mildred Lake reference fish against fish held in dechlorinated tap water at the University of Alberta six times, for a total of 60 judgements. As well, Mildred Lake reference fish were compared against fish initially held in Mildred Lake water and then depurated (if needed) for 24 hours in Syncrude dechlorinated tap water.

Sensory evaluation required three separate sessions, on three separate days, at approximately the same time each day. Panelists were advised to refrain from eating or drinking for at least 30 minutes prior to the sessions. As well, they were discouraged from wearing heavy perfumes or using scented soap on the day of a sensory evaluation session (Larmond 1977).

4.4 WATER AND WASTEWATER ANALYSIS

Water temperature, pH, and conductivity were recorded immediately before and after the exposure period.

Immediately prior to fish exposure, wastewater samples were collected in 4 L amber bottles with teflon-lined caps. Each sample was preserved by adding 100 mL DCM and stored at 2 to 4 ^{o}C, awaiting solvent extraction.

4.4.1 Base-neutral Solvent Extraction Based on Method 625, U.S. EPA (1982), six mL of 6N NaOH was added to raise

the pH of a 4 L water sample to pH 10 or above. An additional 100 mL DCM solvent was added. The sample was stirred for 30 minutes on a magnetic stirrer. After a 20 minute settling period the DCM layer containing the base-neutral extractable organics was removed by pipette. The extract was passed through a (drying) column containing 50 grams Na_2SO_4 in order to remove any water contamination. (The Na_2SO_4 had been pre-extracted with DCM solvent in order to remove any organic contaminants). Another 100 mL DCM was added to the 4 L water sample and the same procedure repeated.

4.4.2 Acid Extraction Following extraction at high pH (Section 4.4.1), the same water sample was acidified to below pH 2 by the addition of 12 mL 6N H_2SO_4. DCM (100 mL) was added and the sample stirred for 2 hours. After settling, the DCM layer was collected and passed through a clean, pre-extracted Na_2SO_4 column. The procedure was repeated a second time.

Both base/neutral and acid extracts were reduced to 6 mL in a Kuderna-Danish (KD) apparatus heated by a waterbath maintained at 65 ^{o}C, following the method outlined in U.S EPA (1982). The extracts were finally reduced to 1 mL under a stream of nitrogen.

All extracts were analyzed by GC-FID, GC-FPD, and by GC-MS. (See section 4.8 for instrument specifications and operating conditions).

4.5 BILE ANALYSIS

4.5.1 Direct Injection to Liquid Chromatograph

Following a method adapted from Krahn et al. (1984), 5 µL samples of MLK, TTP, and UA raw fish bile were injected directly into HPLC.

Samples were injected using a Waters model 710B auto-injector (Milford, MA, USA). Solvent delivery was provided by two Waters model 6000A chromatography pumps. Solvent selection and regulation was performed by a Waters Automated Gradient Controller. The analytical column was a 150 mm by 4.6 mm I.D., reverse phase, LC-PAH packed column (Sulpelco Inc., Bellefonte, PA, USA).

Water (solvent A) and methanol (solvent B) were used in a linear gradient as follows: 100% solvent A to 100% solvent B in 15 minutes; 10 minutes at 100% B; and 3 minutes to return to 100% A. Solvent flow was 1 mL per minute. All analyses were performed at ambient temperature. Chromatograms were recorded at fluorescence excitation/emission wavelengths of 254/375 nm, respectively.

4.5.2 Enzymic Hydrolysis of Bile Metabolites Using a method described by Krahn et al. (1984), beta-glucuronidase (0.1 mL, 2000 units glucuronidase activity and 25 units sulphatase activity) was dissolved in acetate buffer (0.4 M acetic acid, 0.4 M sodium acetate, pH 5, 1 mL), and added to 200 µg of preweighed fish bile. The mixture was placed in a shaking incubator at 37 °C for 16 hours to hydrolyze the conjugates (Varanasi et al. 1982).

The extraction procedure, a modification of Baird et al. (1977) and Varanasi et al. (1982) consisted of the following:

1. Volumes of 2.5 mL methanol, and 1.25 mL chloroform were added to the hydrolyzed mixture, followed by 1 minute of vigorous mixing on a vortex machine.
2. An additional 1.25 mL chloroform was added, followed by 1 minute of vigorous mixing.
3. As a final wash, 1.25 mL water was added, followed by 1 minute of vigorous mixing.
4. The layers were allowed to separate, and the lower chloroform layer removed and passed through a pre-extracted Na_2SO_4 drying column.
5. An additional 2 mL chlororform was added to the remaining aqueous phase, and vigorously mixed for 1 minute.

6. The chloroform layer was again removed and passed through Na_2SO_4. The Na_2SO_4 column was finally rinsed with 1.25 mL chloroform.

7. The chloroform extract was concentrated down to 1 mL under a stream of nitrogen.

Enzymic hydrolysis was carried out in triplicate on bile from each type of tailings pond water fish exposure. All extracts were analyzed by GC-FID/FPD. Those revealing the presence of xenobiotics were then analyzed by GC-MS.

4.6 TISSUE PROCESSING

4.6.1 Blending and Soxhlet Extraction Fish were prepared according to the method reported by Benville and Tindle (1970). By this method, semifrozen fish fillets were chopped into 10 mm by 10 mm pieces, added to a Waring blender with an equal amount of dry ice pellets, and pulverized into a homogenous mixture. The mixture was poured into glass containers, covered with aluminum foil and left over-night in a freezer, allowing the dry ice to sublime.

In order to bind tissue-held water, 20 g samples of blended fish tissue were mixed with 80 g of Na_2SO_4, as in the procedure described by Hesselberg and Johnson

(1972); and added to glass thimbles. To protect the porous plate at the bottom of the glass thimble from getting plugged with fish lipid matter, pre-extracted celite was first added to a depth of 20 mm. The tissue/Na_2SO_4 mixture was added, and covered with a plug of pre-extracted glass wool. The prepared thimble was then placed in a Soxhlet apparatus and refluxed for 8 h with 300 mL DCM. The extract was reduced in volume to approximately 8 mL in a KD apparatus held in a water bath maintained at 65 ^{o}C. The extract contained fish lipids and, if present, hydrocarbons originating from the tailings pond.

4.6.2 GPC Separation Gel permeation chromatography (GPC) separation occurs due to differences in molecule size. In this project, GPC was used to separate fish lipids from volatile hydrocarbons present in the soxhlet extracts. The molecular weights of most volatile hydrocarbons are between 75 and 300, while those of most lipids are between 600 and 1500. The procedure was adapted from Stalling et al. (1972), and later modified by Birkholz et al. (in prep).

GPC was performed using 600 mm by 19 mm I.D., chromatography columns. The columns were prepared by placing a plug of pre-extracted glass wool at the bottom

of the column. Bio-beads (S-X3) were soaked over-night in a one-to-one solvent mixture of DCM/hexane; then wet-packed to a depth of 500 mm in the GPC columns, and topped with a 20 mm protective layer of sand.

Bio-beads are made of neutral, porous styrene-divinylbenzene copolymer beads. Their pore dimensions depends on the polarity of the eluant employed. In this case, ideal biobead size for best separation of lipid from hydrocarbons was achieved by using a solvent mixture of equal proportions hexane and DCM.

GPC separation involved the following steps:

1. The DCM/hexane solvent was drained into the sand.
2. The concentrated 8 mL soxhlet extract was brought up to 10 mL with DCM, added to the top of the GPC column, and drained into the sand.
3. The K-D apparatus was rinsed with 10 mL DCM/hexane; this was added to the GPC column, and once again drained into the sand.
4. A reservoir containing 230 mL DCM/hexane was placed on top of the GPC column, and draining was continued.
5. On the basis of previous recovery trials, the first 90 mL was collected and discarded since it contained unwanted fish lipid material.

6. The next 80 mL was collected, and reduced to 8 mL in a KD apparatus held in a water bath at 80 °C.

4.6.3 Florisil Cleanup After GPC separation, florisil was used to remove the final remnants of fish lipid material. Florisil was prepared using a procedure adapted from Hesselberg and Johnson (1972). Samples of florisil (10.0 g) were added to 25 mL glass scintillation vials, and placed in a muffle furnace at 400 °C for 4 hours in order to remove volatile contaminants. Afterwards the florisil was cooled in a desiccator. Plastic caps lined on the inside with aluminum were then tightly screwed over the glass containers. To avoid unwanted entry of atmospheric moisture, the caps were wrapped with teflon tape.

For florisil column preparation a pre-extracted, glass wool plug was placed at the bottom of a 400 mm by 10 mm I.D., glass chromatography column. Florisil (10.0 g) was wet-packed in the column using hexane. A 20 mm layer of Na_2SO_4 was added on top of the florisil to protect against water contamination.

Florisil cleanup involved the following steps:

1. Hexane was first drained into the Na_2SO_4 layer.

2. The 10 mL GPC extract was added and drained into the Na_2SO_4 layer.

3. Step 2 was repeated with an additional 10 ml hexane derived from the KD apparatus rinse.

4. An additional 40 mL hexane was added to the 100 mL reservoir at the top of the column, and drained into the Na_2SO_4. All 60 mL of added hexane was collected.

5. DCM (50 mL) was then added to the same column, drained through, and collected.

6. Both the hexane and DCM fractions were reduced to 8 mL in a KD apparatus, and reduced further to 0.5 mL under a stream of nitrogen. Both fractions were analyzed by GC-FID, GC-FPD, and where appropriate, by GS-MS.

Florisil cleanup separates compounds on the basis of their polar character, retaining either polar or nonpolar substances depending on the polarity of the eluant being used. When a nonpolar solvent is used, florisil must first be partially deactivated by adding a certain percentage of water, otherwise it will adsorb all organic compounds that have been added to the column. In this project florisil was deactivated by either 5 or 10% water, depending on adsorption characteristics of the florisil batch used.

4.7 QUALITY CONTROL - RECOVERY EFFICIENCY

For the wastewater extraction procedure, quality control was performed by the addition of a 20 µg/mL alkylated benzenes standard, and a 20 µg/mL alkylated phenols standard to two 4 L samples of distilled water, followed by base-neutral and acid extraction (described in section 4.4). The extracts were injected into GC-FID and the resultant chromatogram peak areas compared to those of a benzene or phenol standard to determine percent recovery.

For the bile enzymatic hydrolysis, quality control was performed by using the same hydrolysis procedure described in section 4.5.2 on 100 µL of 2 mg/mL 1-naphthyl glucuronide, and determining percent recovery by GC chromatogram peak-area calculations. In addition, to determine the effect (if any) of the buffer alone on bile, bile suspected to contain conjugated hydrocarbon metabolites was first hydrolyzed with buffer only, extracted, hydrolyzed for a second time with buffer and glucuronidase enzyme, and again extracted. Both the first and second extracts were analyzed by GC-FID.

For the tissue extraction process, quality control was performed by the addition of: a) 1 mL of a 20 µg/mL alkylated benzenes standard to two clean control

fish samples; and b) 1 mL of 20 µg/mL alkylated naphthalenes, benzothiophene, and dibenzothiophene standard to one clean fish sample, - in each case immediately prior to Soxhlet extraction. After going through the Soxhlet, GPC, and florisil processes, the extracts were injected into GC-FID and percent recovery calculated from the resultant chromatogram peak areas.

To determine what percent deactivation to use on the florisil, four columns were set up; two with 5% deactivated florisil, and two columns with 10% deactivation. To one pair of 5 and 10% deactivated columns, 1 mL of the 20 µg/mL alkylated benzenes standard was added; to the second pair, 1 mL of the 20 µg/mL alkylated phenols standard was added. Hexane and DCM were added, collected, and concentrated as decribed in section 4.6.3. Maximum percent recovery of the two standards, based on GC-FID chromatogram area calculations, determined the percentage deactiviation used.

4.8 GC INSTRUMENTS AND CONDITIONS

Bile, tissue, and wastewater extracts were all analyzed by GC-FID, GC-FPD, and GC-MS. In each case a 2 µL aliquot of the extract was removed using a Hamilton 10 µL syringe (Hamilton Co., Reno, NV, USA). When using the FPD and FID detectors, the 2 µL aliquots were injected

into a 30 m x 0.25 mm I.D., fused silica, capillary column, with a DB-5 coating (J & W Scientific, Folsom, CA, USA). When the mass spectrometer was used, the 2 µL aliquots were injected into an HP-1 capillary column, 12 m x 0.2 mm I.D. (Hewlett-Packard, Palo Alto, CA, USA).

The flame photometric detector operated in a Varian (Walnut Creek, CA, USA) model 3500 capillary gas chromatograph. The FPD performed at a hydrogen flow rate of 80 mL/minute, and flow rates of 80 mL and 175 mL/minute for air #1, and air #2, respectively. The oven temperature program was 40°C for 1 minute; 10°C/minute to 300°C; and held at 300°C for 15 minutes. Injection port, and detector temperatures were 270°C and 300°C, respectively. Peak areas were measured using a Varian model DS604 data system.

Two flame ionization detectors were used. One of the detectors was in a Hewlett-Packard model HP 5880A series gas chromatograph with HP 5880A computing integrator for measuring peak areas. The other FID was in a Varian model 3550 gas chromatograph described previously. The oven temperature program, and injection port and detector temperatures were identical to those used with the FPD. Gas flow rates were 24, 20, and 280 mL/min for hydrogen, nitrogen and air, respectively.

There were two temperature programs for the GC-MS system. The first program, designed for separating a wide variety of aromatics, was identical to that for the GC-FID/FPD procedures. The second program was more specific for separating phenols. The oven temperature program for separating phenols was: 40°C for 1 minute, 6°C/minute to 280°C, with no temperature hold.

GC-MS was performed with a Hewlett-Packard model HP 5890A gas chromatograph, connected with a model HP 5970 series mass spectrometer, and an HP 7946 data system.

4.9 CHEMICALS

Solvents were either pesticide grade, or HPLC grade. Methanol, acetone, methylene chloride, hexane, and chloroform were purchased (and used interchangeably) from Fisher Scientific Co. (Fairlawn, NJ, USA); Caledon Laboratories Ltd. (Georgetown, ON, Can.); and from Anachemia Chemicals Inc. (Champlain, NY, USA).

Acetic acid (glacial), sulphuric acid, and sodium hydroxide, all reagent grade quality, were purchased from Fisher Scientific Co. Florisil (60 -100 mesh), celite (545), sodium sulphate (anhydrous) were also purchased from Fisher Scientific Co. Anhydrous sodium acetate was purchased from J.T. Baker Chemical Co. (Phillipsburg, N.J., USA). For gel permeation chromatography, Bio-beads

S-X3 were purchased from Bio-Rad Laboratories Inc. (Richmond, CA, USA). For bile analysis, ß-glucuronidase No. G-8132, and 1-naphthyl glucuronide were purchased from Sigma Chemical Co. (St Louis, MO, USA).

The following compounds were purchased from Dixon Chemicals Co. (Edmonton, AB,) to make up an alkylated phenol standard: phenol; 2, and 3, and 4-methyl phenol; 2,3 and 2,4 and 2,5 and 2,6-dimethyl phenol. An alkylated benzene standard was made by purchasing the following compounds from PolySci Corporation: ethyl benzene; 2, and 3 methyl benzene; 1 methyl ethyl benzene; n-propyl benzene; 1,3,5-trimethyl benzene; 4-isopropyl toluene; and n-butyl benzene.

5. RESULTS AND DISCUSSION

5.1 SENSORY EVALUATION OF EXPOSED FISH

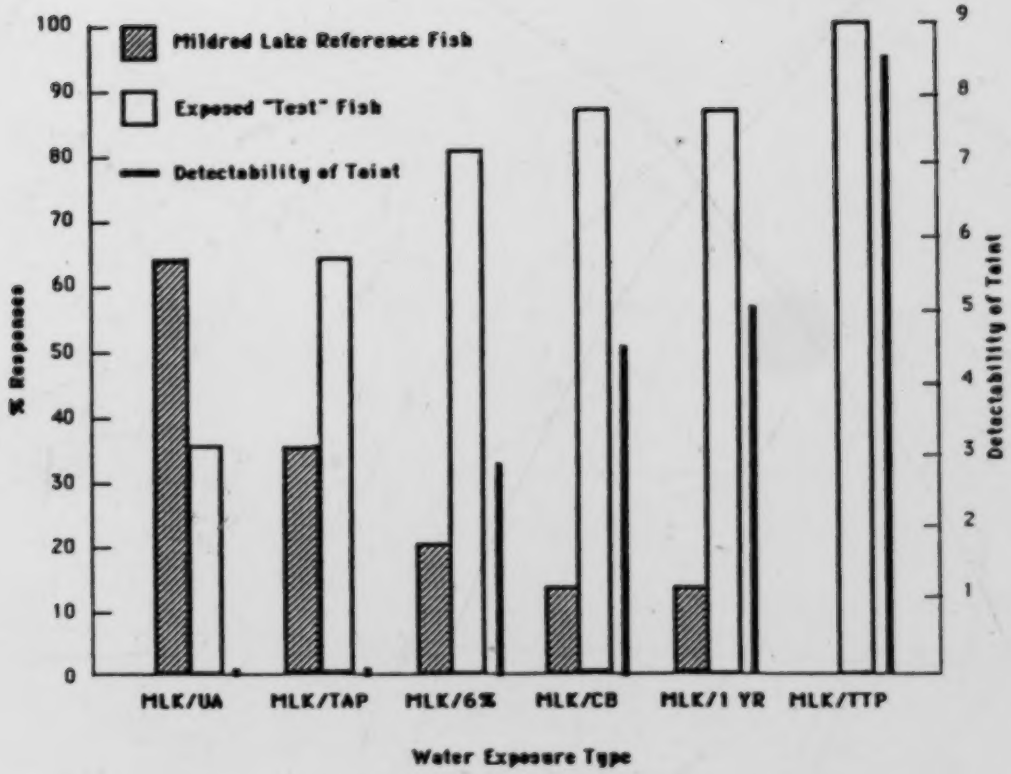
Oil sand extraction tailings pond waters contain petroleum compounds and inorganic compounds, some of which are different, and others which are similar but in different relative concentrations to those found in oil refinery effluents elsewhere. Because of these differences and because of the possiblity of unknown synergistic effects, it is not possible to directly transcribe tainting results from studies performed at conventional oil refineries (Krishnaswami and Kupchanko 1969) or those performed with pure organic compounds (Ogata and Miyake 1973), to the effect of oil sands extraction tailings pond waters on fish tainting.

The results of the sensory evaluation performed on fish exposed to tailings pond waters are found in Table 3, and Figure 7, with the supporting data found in Table 16, Appendix I. According to the two-tailed test table provided in Roessler et al. (1956), (Table 17, Appendix I), a significant difference in fish taint occurs, at a 5% significance level, if a minimum of 21 out of 30 evaluations, or 39 out of 60 evaluations choose one fish exposure type or the other as being more tainted. Table 17 was constructed for binary situations, in this investigation - one fish sample being more or less tainted

Table 3. Sensory analysis results of fish exposed to oil sands extraction tailings pond wastewaters, using the "paired comparision" test.

Exposure water	Number of judgements choosing one or the other as more tainted	Percent responses	Average detectability of taint, maximum = 9
UA[a]	22	37	
- compared to			0.7
MLK[b]	38	63	
TAP[c]	19	63	
- compared to			1.1
MLK	11	37	
6%[d]	24	80	
- compared to			3.1
MLK	6	20	
CB[e]	26	87	
- compared to			4.6
MLK	4	13	
1YR[f]	26	87	
- compared to			5.1
MLK	4	13	
TTP[g]	30	100	
- compared to			8.7
MLK	0	0	

[a]University of Alberta dechlorinated tap water. [b]Mildred Lake water. [c]Dechlorinated tap water at Syncrude. [d]Six percent whole tailings pond water. [e]Catchment basin water. [f]One year stored tailings pond water. [g]Treated tailings pond water.



Detectability of Taint Scale: 1 - barely detectable
9 - highly detectable

MLK = Mildred Lake (holding and reference) water
UA = University of Alberta tap water
TAP = Tap water at Syncrude
1 YR = One year stored (aged) tailings pond water
6% = 6% tailings pond water
CB = Tailings pond catchment basin water (rainwater & dyke drainage water)
TTP = Treated tailings pond water

Figure 7. Percent of sensory panel responses choosing either fish exposed to a test water, or to Mildred Lake water (only) as being more tainted; plus the level of detectability of the evaluated taint.

than the other. An analogy can be made to flipping a coin. 95% of the time a coin is flipped, the ratio of heads to tails will be between 0.3 (9/30) and 0.7 (21/30). Based on the two-tailed test in Table 17, no significant difference in taint ($P<0.05$) was found when fish exposed to Mildred Lake (MLK) water were compared with those fish exposed either to Mildred Lake followed by 24 hours in dechlorinated Syncrude tap water (TAP), or those held in dechlorinated Edmonton tap water at the University of Alberta (UA).

Based on the one-tailed test (or two-tailed test) found in Table 17, fish first held in Mildred Lake water and then exposed for 24 hours to various tailings pond derived waters were all significantly tainted ($P<0.05$) when compared to fish held in Mildred Lake water only.

Fish held in one-year stored tailings pond water (1YR) started to die within 2.5 hours of exposure because the water was unexpectedly toxic. The remaining living fish were therefore sacrificed at this time. Sensory evaluation revealed that this short exposure period still caused a significant taint ($P<0.05$). According to MacKinnon and Boerger (1986) the LC_{50} (96 hour) for trout in one-year stored tailings water is 100%. According to Boerger (personal communication) when one-year water was

pumped from the main storage pond to the fish exposure pool, sludge from the bottom of the storage pond was likely included with the water, which may have caused the observed toxic effect.

During the sensory evaluation sessions the panelists were required to indicate the level of the detectable taint. The scale provided was from 1-9 with 1 indicating "barely detectable" and 9 indicating a "highly detectable" taint. The "average" detectability of taint was determined by subtracting the sum of detectability scores given by the minority of judgements (for example, those choosing MLK as being more tainted than CB) from the sum given by the majority of judgements; and then dividing by the total number of judgements. This would be similar to subtracting wrong answers from right answers on a test, and then taking the average.

Figure 7 illustrates the relationship between detectability of taint and percent responses. According to the "percent responses" and "detectability of taint" scores, treated tailings pond caused the greatest taint followed by one-year stored tailings, catchment basin, and finally six percent whole tailings pond water.

5.2 WATER AND WASTEWATER ANALYSIS

5.2.1 Gross Physical and Chemical Parameters. Table 4 provides chemical and physical parameters of the test waters, excluding Mildred Lake and University of Alberta exposure waters, which were not analyzed.

Temperature of the wastewaters remained constant (within 1 ^{o}C) throughout the fish exposure period since the exposure pools were resting on a concrete floor in a warehouse whereby the pools adjusted to the constant temperature of the floor.

Each wastewater was continuously aerated, resulting in oxygen saturated conditions throughout the 24 hour exposure period. Aeration was performed to ensure survival of the test fish since it would have been difficult to find replacements if the test fish had died during the 24 hour exposure period. Even with aeration and the potential resultant loss of volatile petroleum compounds (capable of tainting) from the exposure waters, a significant taint ($P<0.05$) was noted.

According to Boerger et al. (1984) conductivity of whole tailings pond water (TP) was 1410 µS/cm; for treated tailings pond water (TTP) was 1790 µS/cm (note: TTP was more than TP); and for 10 month stored tailings pond water was 860 µS/cm. According to MacKinnon (1986)

Table 4. Temperature, conductivity and pH of the fish exposure waters.

	TAP	6%	CB	1YR	TTP
Temperature (oC):					
Pre-exposure	15.7	15.4	16.4	17.1	15.6
Post-exposure	16.0	15.5	16.0	n/a[a]	15.6
Conductivity (µS/cm):					
Pre-exposure	326	435	1660	3060	2530
Post-exposure	339	423	1596	n/a	2520
pH:					
Pre-exposure	6.9	7.1	8.2	7.9	7.2
Post-exposure	8.5[b]	7.8	8.3	n/a	8.2

[a]Non-applicable, since the fish were removed from this exposure water after just 2.5 hours as compared to 24 hours for the other exposure waters.
[b]Variation in pH may be due to the use of an uncalibrated pH meter.

conductivity of tailings pond water was recorded at 1780 µS/cm, an increase over the figure reported in 1984. Based on these reports the figure for treated tailings pond water, 2520 µS/cm, recorded in Table 4 is comparable.

Conductivity recorded for one-year stored tailings pond water in this study was more than 3.5 times the level reported by Boerger et al. (1984), which provides some support to Boerger's speculation (personal communication) that sludge from the bottom of the storage pool may have affected the toxicity of the one-year stored water exposure.

A complete characterization of the wastewaters was not carried out to determine what substances were contributing to conductivity , pH, toxicity, etc. Some of this information may be obtained in Boerger et al. (1984), where extensive physical and chemical properties of fresh tailings pond water, treated tailings pond water and stored tailings pond water is presented, albeit for 1984. Included are dissolved and suspended solids; biological and chemical oxygen demand; major ions such as sodium, calcium, potassium and sulphate; alkalinity; total organic and inorganic carbon; nitrogen; phosphate; cyanide; and minor elements such as aluminum, iron and nickel.

5.2.2 Alkylated Phenols. Table 5 provides the concentrations of phenols found in both the acid and base extracts. Phenols are more readily extracted under acidic conditions, however from Table 5, and the percentage recovery figures presented in Table 6, it is clear that some phenols are also extracted under base-neutral conditions. Zenon (1984) also found alkylated phenols in the base-neutral fraction of whole tailings pond.

Recovery of alkylated phenols by solvent extraction (Table 6) was much lower than recovery of alkylated benzenes (Table 8). Table 2 provides the water solubilities of various petroleum compounds. From Table 2 it can be seen that alkylated phenols are far more water soluble than alkanes, alkylated benzenes, PAH's, and organic sulphur compounds. Being more water soluble (more polar) likely contributed to the lower recovery of alkylated phenols as compared with alkylated benzenes using the solvent extraction method.

Other problems exist with the solvent extraction method used. According to EPA (1982), the authors of this method, base-neutral extraction may significantly reduce recovery of phenol, 2-cresol, and 2,4-dimethyl phenol. Secondly, based on analysis of centrifuged and non-centrifuged whole tailings pond water, Zenon (1984) found the extractability of organics was

Table 5. Alkylated phenols (µg/L) in treated tailings pond water (TTP).

	Base-Neutral Extract	Acid Extract	Combined Extract
phenol	7.1	13.2	20.3
2-cresol	N.D[a]	43.5	43.5
3 and/or 4-cresol	3.9	31.0	34.9
2,3 and/or 3,5-dimethyl phenol	N.D.	36.8	36.8
2,4 and/or 2,5-dimethyl phenol	12.5	33.8	46.3
2,6-dimethyl phenol	2.8	10.0	12.8
3,4-dimethyl phenol	6.8	11.0	17.8
Total -			
phenol	7.1	13.2	20.3
cresols	3.9	74.5	78.4
dimethyl phenols	22.1	91.6	113.7
Total phenols	33.1	179.3	212.4

[a]Not detected.

Table 6. Percent recovery of alkylated phenols which had been added to a water sample and then solvent extracted under base-neutral and acid conditions.

	% Recovery		
	Base/neutral	Acid	Combined
phenol	0	24	24
2-cresol	5	62	67
2,6-dimethyl phenol	42	46	88
3-cresol	10	53	53
4-cresol	4	55	59
2,4 and 2,5-dimethyl phenol	17	74	91
2,3 and 3,5-dimethyl phenol	13	74	87
3,4-dimethyl phenol	9	75	84
Average recovery	11	58	69
Summary:			
Recovery of phenol			24
Average recovery of 3 cresols			60
Average recovery of 4 dimethyl phenol peaks			88

impeded by the presence of suspended material in the non-centrifuged tailings pond sample.

Figure 8 provides a comparison of an acid extract GC-FID chromatogram produced in this project with that of MacKinnon (1984); GC conditions may not be the same. The GC-FID peak times and areas from the TTP acid extract (top chromatogram), combined with the peak times and areas of an alkylated phenol standard found in Figure 9 were used to calculate the phenol concentrations recorded in Table 5. Table 7 illustrates the variability, and the constancy of GC-FID retention times of an alkylated phenols standard injected manually.

Figures 10 and 11 provide GC-MS evidence of the presence of alkylated phenols identified in the GC-FID TTP acid chromatogram.

MacKinnon (1986) identified 130 µg/mL phenols in treated tailings pond water. The concentration reported in Table 5, (212 µg/mL), calculated in this project is higher, yet comparable.

No literature was found reporting fish tainting to occur at the low wastewater phenol concentrations identified in this project.

5.2.3 Alkylated Benzenes. Alkylated benzenes are found in the base-neutral extract, as illustrated by the

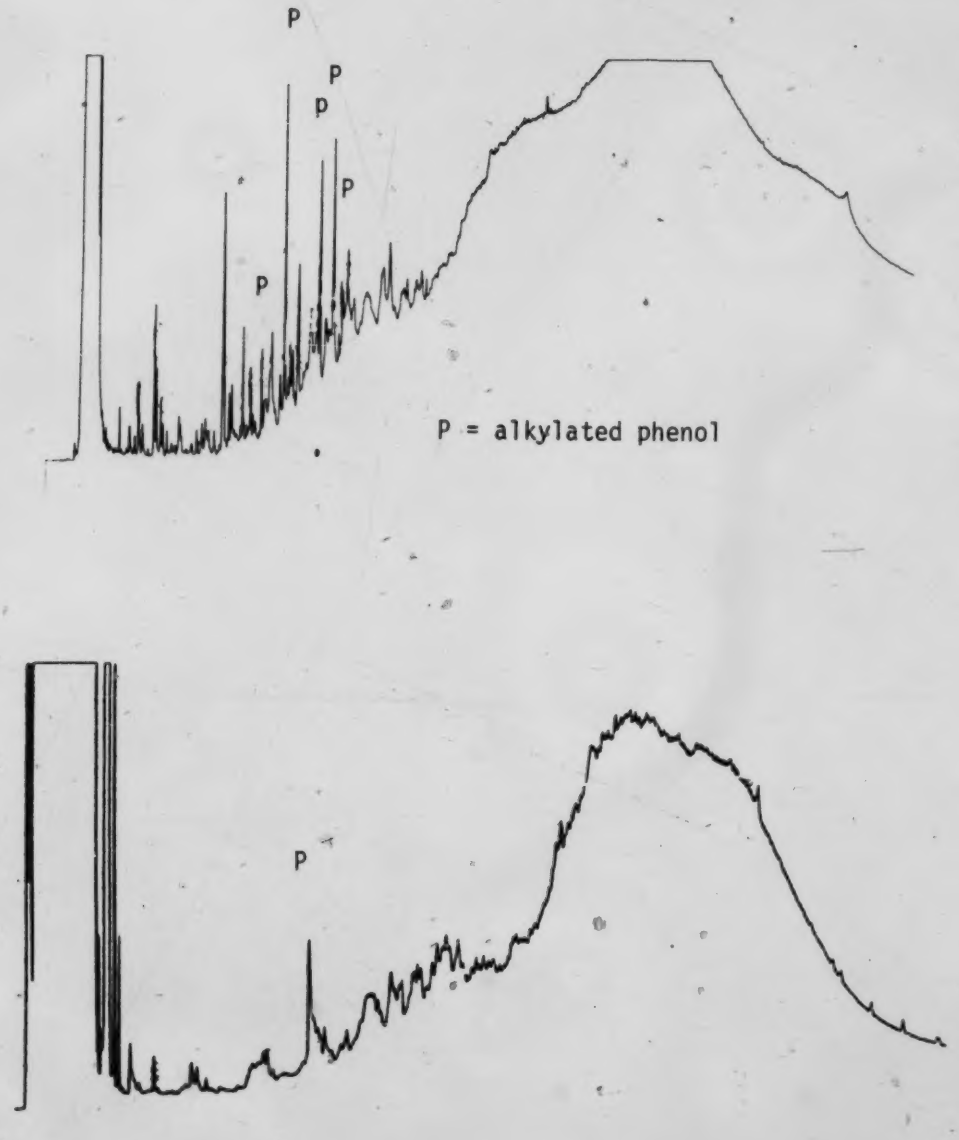
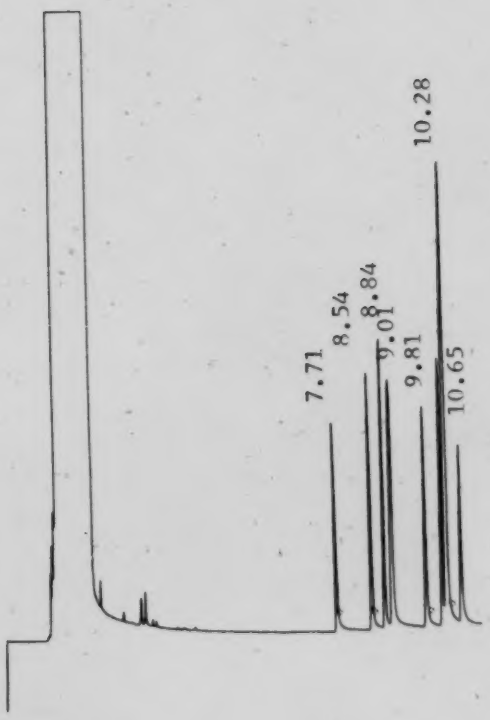


Figure 8. Comparison of two GC-FID chromatograms of acid extracts from treated tailings pond water; top - from this project, at 10% of full strength; the bottom - adapted from MacKinnon (1984).



Time (min)	Compound
7.71	phenol
8.54	2-cresol
8.84	2,6-dimethyl phenol
9.01	3 or 4-cresol
9.81	2,4 or 2,5-dimethyl phenol
10.28	2,3 or 3,5-dimethyl phenol
10.65	3,4 dimethyl phenol

Figure 9. GC-FID chromatogram of an alkylated phenol standard.

Table 7. GC-FID retention times of phenols in an alkylated phenol standard.

	Mar 18	Mar 23	Mar 24	Mar 25	Mar 26	Mar 27
phenol	7.74	7.71	7.70	7.71	7.70	7.69
2-cresol	8.56	8.54	8.53	8.53	8.52	8.51
2,6-dimethyl phenol	8.85	8.84	8.84	8.84	8.83	8.82
3 and 4-cresol	9.03	9.01	9.01	9.03	9.01	9.00
2,4 and 2,5-dimethyl phenol	9.83	9.81	9.81	9.81	9.80	9.79
2,3 and 3,5-dimethylphenol	10.29	10.28	10.28	10.28	10.27	10.26
3,4-dimethyl phenol	10.66	10.65	10.64	10.64	10.63	10.62

	March 23				March 26	
	1	2	3	4	1	2
phenol	7.71	7.71	7.71	7.71	7.70	7.72
2-cresol	8.54	8.53	8.54	8.53	8.52	8.54
2,6-dimethyl phenol	8.84	8.84	8.84	8.84	8.83	8.84
3 and 4-cresol	9.03	9.03	9.01	9.01	9.01	9.01
2,4 and 2,5-dimethyl phenol	9.81	9.81	9.81	9.81	9.80	9.82
2,3 and 3,5-dimethyl phenol	10.28	10.28	10.28	10.28	10.27	10.29
3,4-dimethyl phenol	10.64	10.64	10.65	10.64	10.63	10.65

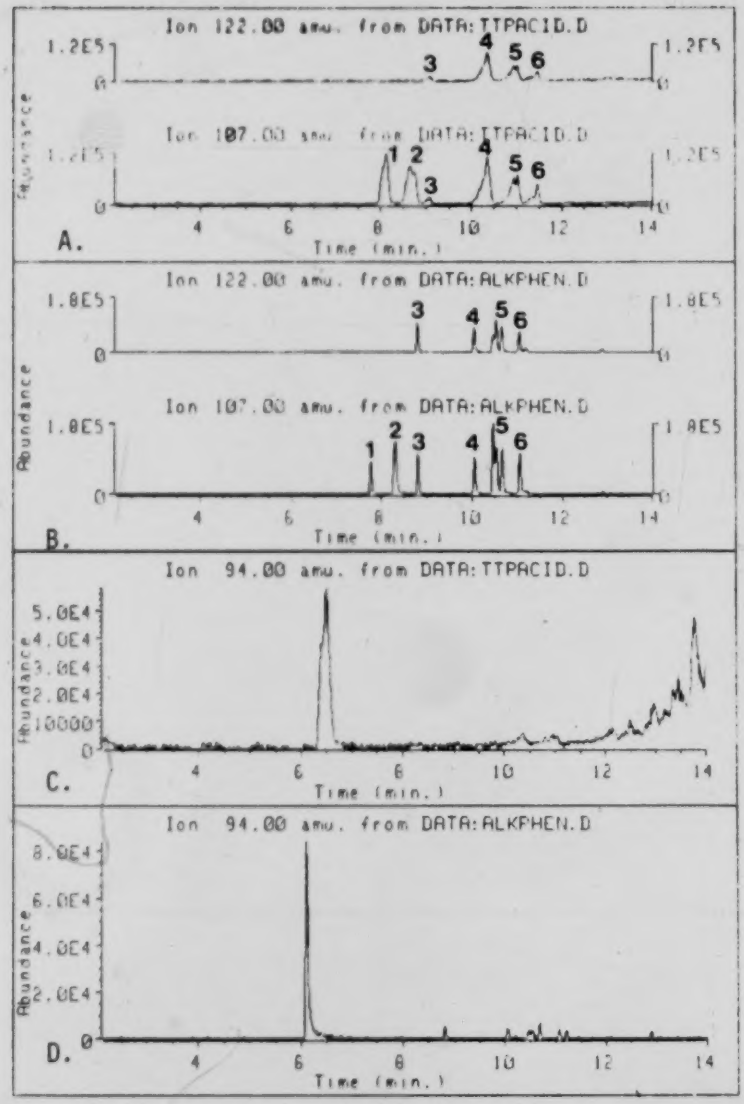


Figure 10. GC-MS selective ion chromatograms. Major ion fragments of cresols (peaks 1,2) and dimethylphenols (peaks 3,4,5,6) in treated tailings pond water acid extract (Box A), and in a cresol and dimethyl phenol standard (Box B). Major ion fragment of phenol (94.00 amu) in treated tailings pond water acid extract (Box C), and in phenol standard (Box D).

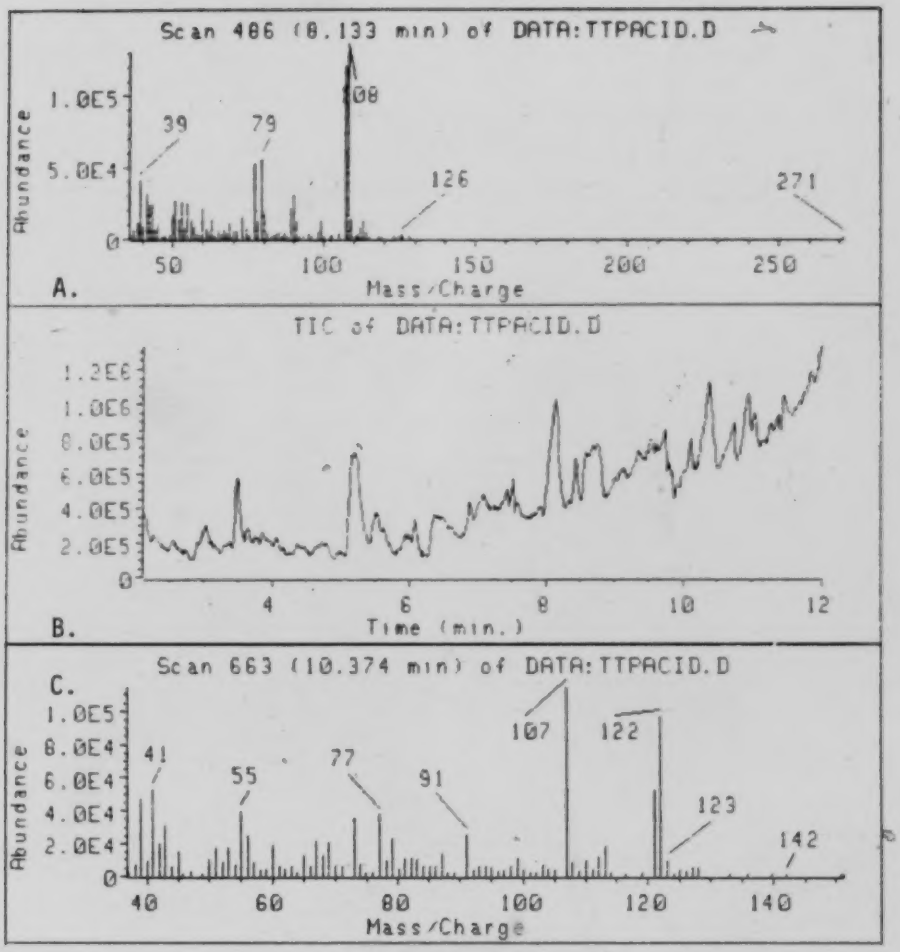


Figure 11. GC-MS results. Box B provides a total ion chromatogram of the first 12 minutes of a treated tailings pond water acid extract injection. A mass spectrum of the peak at time 8.133 min. reveals the presence of cresol (Box A), and dimethylphenol at time 10.374 min. in Box C.

percent recoveries given in Table 8. Table 9 provides the concentration of alkylated benzenes found in treated tailings pond water (TTP). These concentrations are based on comparing peak times and areas found in the TTP base-neutral extract (Figure 12) with those of the alkylated benzene standard (Figure 13).

Figures 14 and 15 provide mass fragments and mass spectra of toluene and xylenes which support the identifications based on GC-FID.

5.2.4 Organic sulphur. Figure 16 provides GC-FPD chromatograms of base-neutral extracts of Mildred Lake and five tailings pond derived waters. An FPD - Flame Photometric Detector was used for identifying polynuclear aromatic sulphur heterocyclics (PASH compounds). Table 10 gives the peak areas, and Table 11, the concentrations of organic sulphur (PASH) compounds derived from the GC-FPD peak areas. Potential tainting compounds such as benzothiophene, dibenzothiophene, and their alkylated derivatives are found in this extract. A correspondence exists between the concentration of organic sulphur in the analyzed waters and the "detectabilty of taint" (9 = highly detectable) noted in fish exposed to tailings pond waters during the sensory analysis sessions.

Table 8. Percent recovery of alkylated benzenes which had been added to a water sample and then solvent extracted under base-neutral and acid conditions.

	% recovery	
	base/neutral	acid
ethyl benzene	86	0
3-xylene	93	0
2-xylene	93	0
1-methyl ethyl benzene	93	0
n-propyl benzene	90	0
1,3,5-trimethyl benzene	92	0
4-isopropyl toluene	92	0
n-butyl benzene	98	0
Average recovery	92	0

Table 9. Alkylated benzenes (µg/L) in treated tailing pond (TTP) water, solvent extracted under base-neutral conditions.

	TTP water
ethyl benzene	0.4
3 and/or 4-xylene	67.1
2-xylene	32.6
1-methyl ethyl benzene	3.8
n-propyl benzene	N.D[a]
1,3,5-trimethyl benzene	6.9
4-isopropyl toluene	44.0
n-butyl benzene	N.D
Total:	
Xylenes only	99.7
All benzenes	154.8

[a]N.D - Not detected.

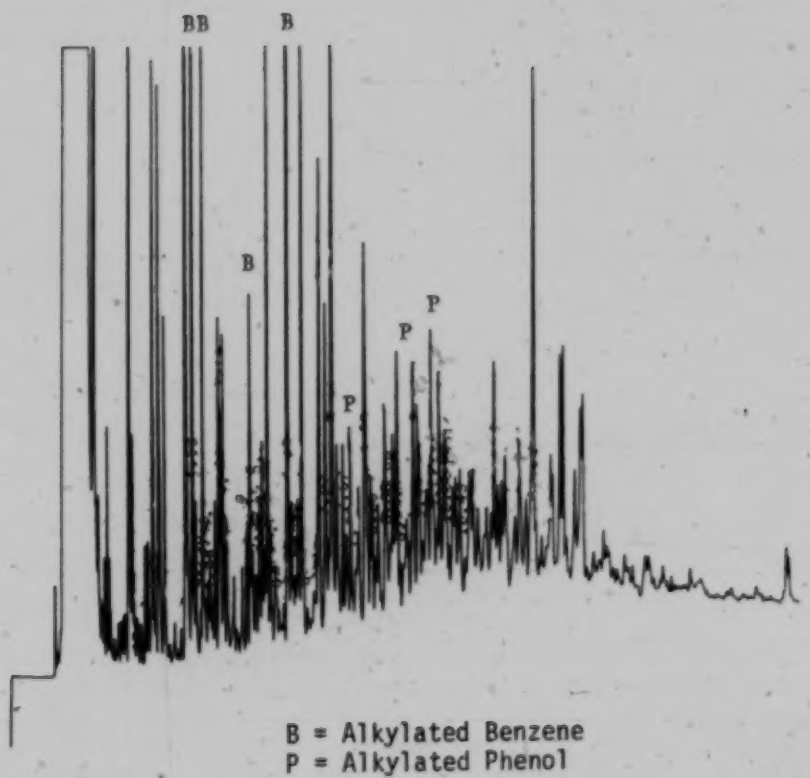
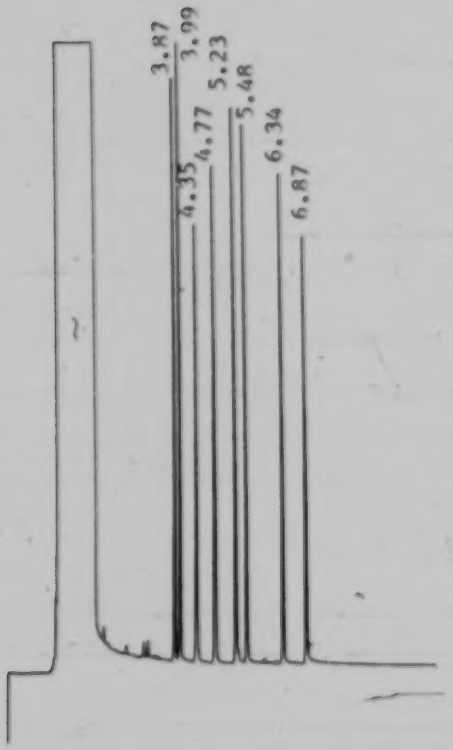


Figure 12. GC-FID chromatogram of treated tailings pond water base-neutral extract (10% of full strength).



Time (min)	Compound
3.87	ethyl benzene
3.99	3 or 4-xylene
4.35	2-xylene
4.77	1-methyl ethyl benzene (cumene)
5.23	n-propyl benzene
5.48	1,3,5-trimethyl benzene (mesitylene)
6.34	4-isopropyl toluene (p-cymene)
6.87	n-butyl benzene

Figure 13. GC-FID chromatogram of an alkylated benzene standard.

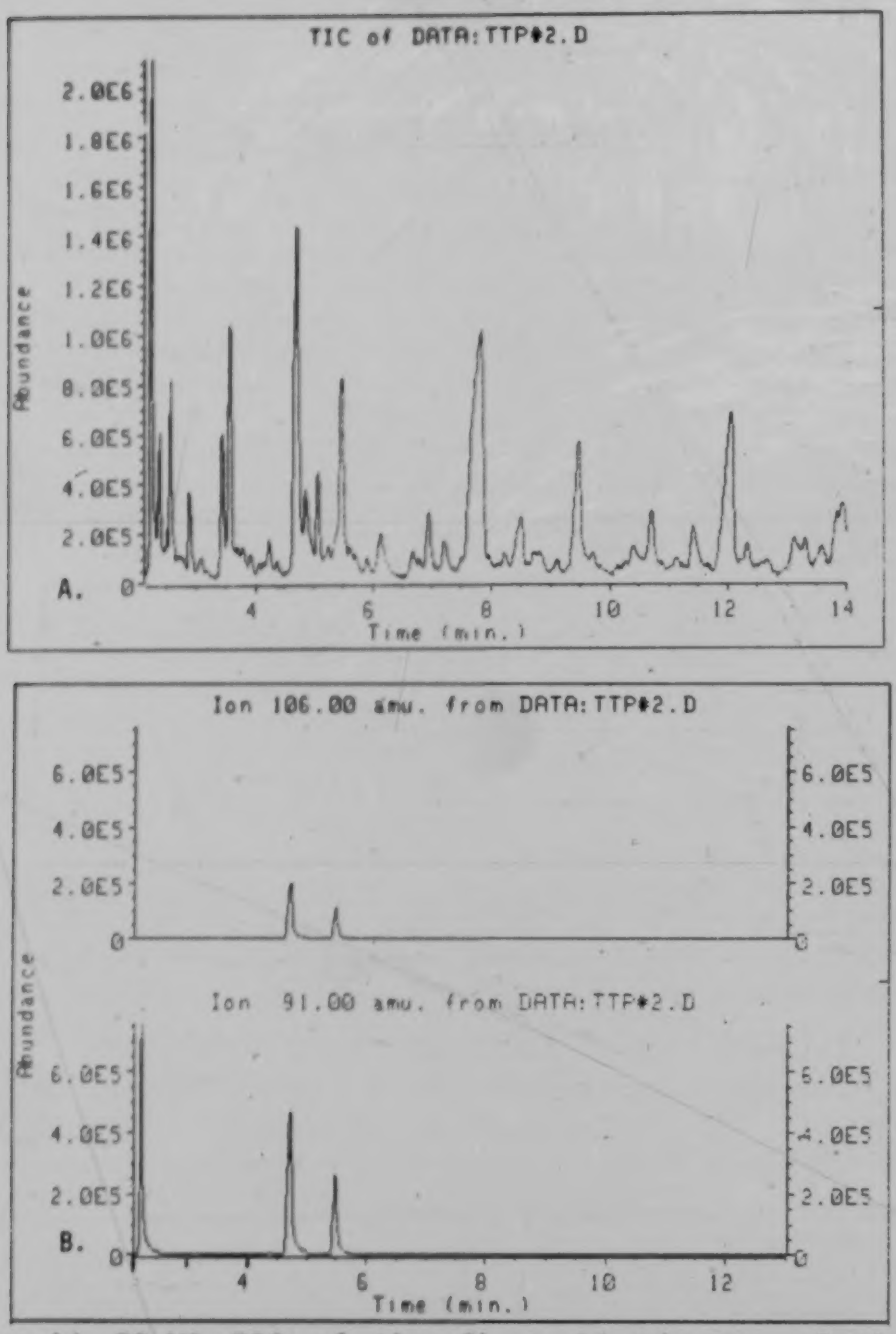


Figure 14. GC-MS TIC of the first 14 minutes of the treated tailings pond water base-neutral extract, (A). Selective ion chromatograms of the major mass fragments of toluene (ion 91) at about 2.5 min., and xylenes (ion 91 and 106) between 4 and 6 min., (B).

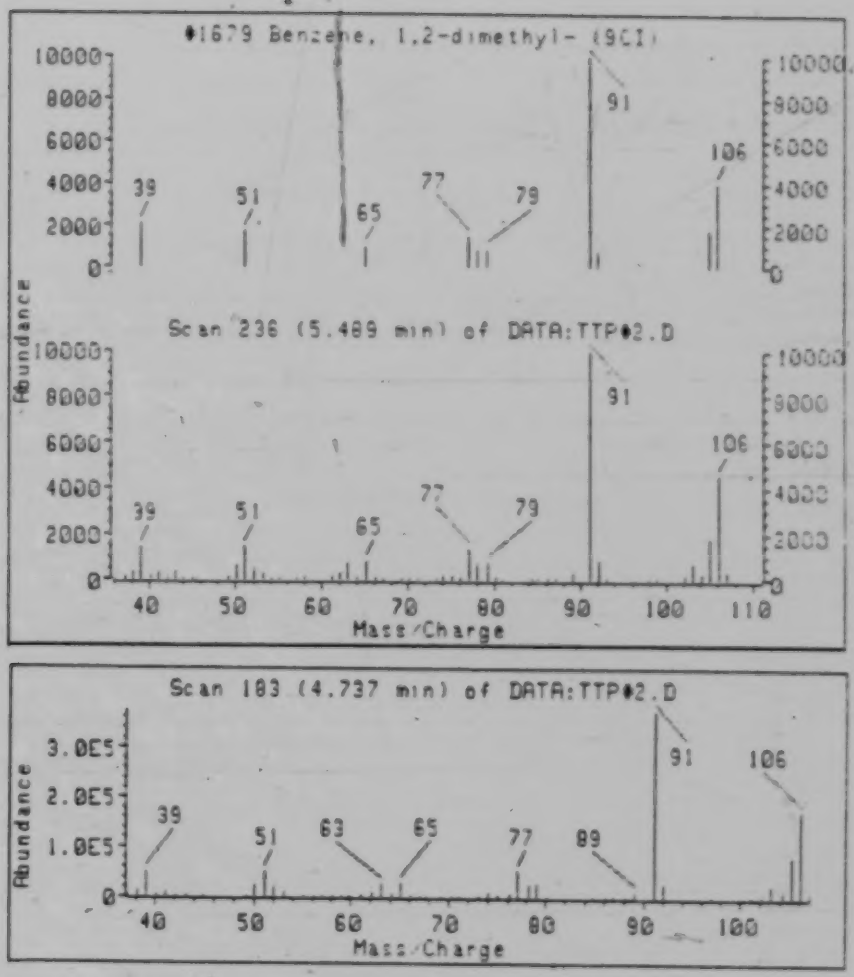


Figure 15. Mass spectra of two peaks found in treated tailings pond water base-neutral extract, revealing the presence of xylenes.

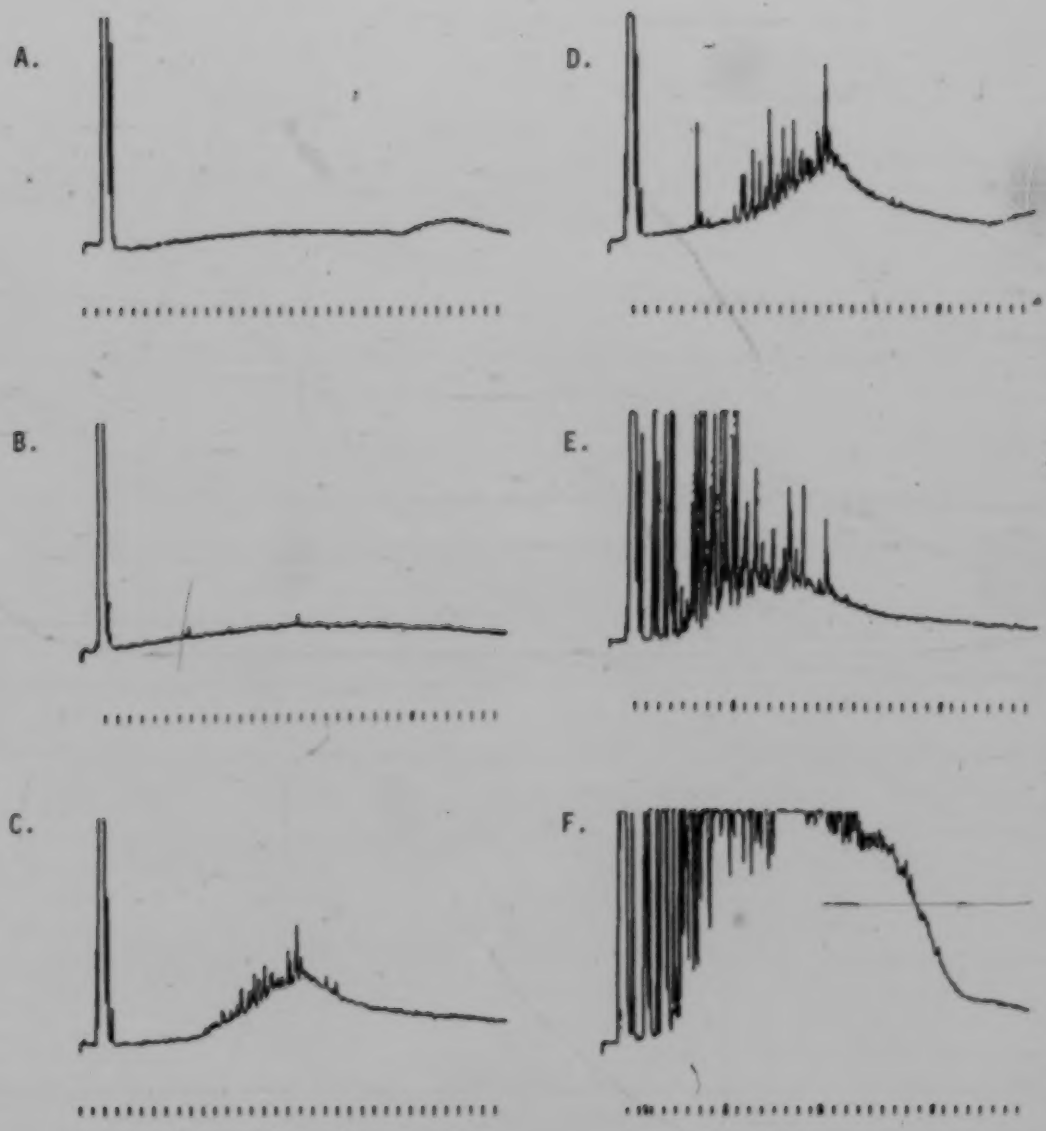


Figure 16. Chromatograms from GC-FPD, for detecting organic sulfur compounds - of base-neutral extracts: A. Mildred Lake water, B. Six percent of whole tailings pond water, C. Catchment basin water, D. One-year stored tailings pond water, E. Treated tailings pond water, F. Whole tailings pond water.

Table 10. Organic sulphur in fish exposure waters (solvent extracted under base/neutral conditions only) as detected by GC-FPD, expressed in GC peak areas.

	Areas from GC-FPD chromatogram peaks (x 104)			
	Peak 1 only[a]	Peak 2 only[b]	Total Area[c]	Detectability of Taint
Tailings pond (TP)	170.0	250.0	17000.0	N.A[d]
Treated TP	56.0	23.0	3600.0	8.7
1 Year Stored	8.0	50.0	1700.0	5.1
Catchment Basin	2.0	33.0	650.0	4.6
6% TP	0.0	7.0	25.0	3.1
Mildred Lake	0.0	2.0	10.0	0.9

[a]Peak 1 - represents the retention time of benzothiophene.
[b]Peak 2 - represents the retention time of dibenzothiophene.
[c]Total Area - represents the total area of all peaks excluding the first 3 min. of gc run to avoid solvent area interference.
[d]N.A = not applicable, no fish exposed to this sample.

Table 11. Organic sulphur compounds (µg/L) in fish exposure waters (solvent extracted under base-neutral conditions), as detected by GC-FPD.

Fish exposure water	Peak 1 only[a]	Peak 2 only[b]	Total Org-S, represent as Bth	Total Org-S, represent as DiBth	Detect. of Taint
Tailings Pond (TP)	2.6	11.0	271.0	783.8	N.A[d]
Treated TP	0.9	1.0	56.8	164.4	8.7
1 year stored TP	0.1	2.2	26.0	75.2	5.1
Catchment Basin	0.03	1.4	10.1	29.1	4.6
6% tailings pond	N.D[c]	0.3	0.4	1.1	3.1
Mildred Lake	N.D	0.1	0.2	0.4	0.9

[a]Represents the retention time for benzothiophene (Bth).
[b]Represents the retention time for dibenzothiophene (DiBth).
[c]Not detected. [d]Not applicable, no fish exposed to this sample.

In Table 11 the total area of the sulphur peaks is decribed in terms of concentrations benzothiophene and dibenzothiophene. This approach of reporting total peak area in terms of a specified compound was used in reporting PAH concentrations in fish bile detected with HPLC- fluorescence by Krahn et al. (1986) and Morgan et al. (1987). In the current investigation, this approach resulted in a three times higher concentration of organic sulphur (PASH) when expressed as dibenzothiophene as compared to expressing it as benzothiophene.

The solvent extraction method used here for measuring concentrations of alkylated phenols and benzenes, and organic sulphur compounds is limited in its ability to retain the more volatile compounds such as phenol, benzene, toluene and thiophene. Trapping and analyzing the volatiles in the space above the wastewaters (purge and trap head-space analysis) may be a more sensitive method, as in Murray et al. (1984).

5.3 BILE ANALYSIS

Bile was analyzed because of its function as a vehicle for the excretion of organic compounds foreign or toxic to the organism. Foreign organic compounds, eg. drugs in humans, or petroleum compounds in fish, are metabolized to less lipid soluble, more water soluble substances by the liver in order to enhance their excretability. The metabolized compounds are then transported from the liver by blood transport to the kidney and excreted with the urine; or transported from the liver by bile to the gall bladder, to the small intestine and excreted with the feces.

5.3.1 HPLC results. As mentioned in Section 3.4.2, HPLC-fluorescence was used by Krahn et al. (1984, 1986) and Malins et al. (1985a, 1985b) to identify a correlation between concentrations of hydrocarbon metabolites in fish bile and the prevalence of fish liver lesions . HPLC bile analysis in this investigation did not reveal a correlation between hydrocarbon metabolites in the bile and the detectability of a petroleum taint in fish.

Figure 17 provides chromatograms of raw bile injected directly into HPLC. The results are inconclusive. Bile extracted from control-trout at

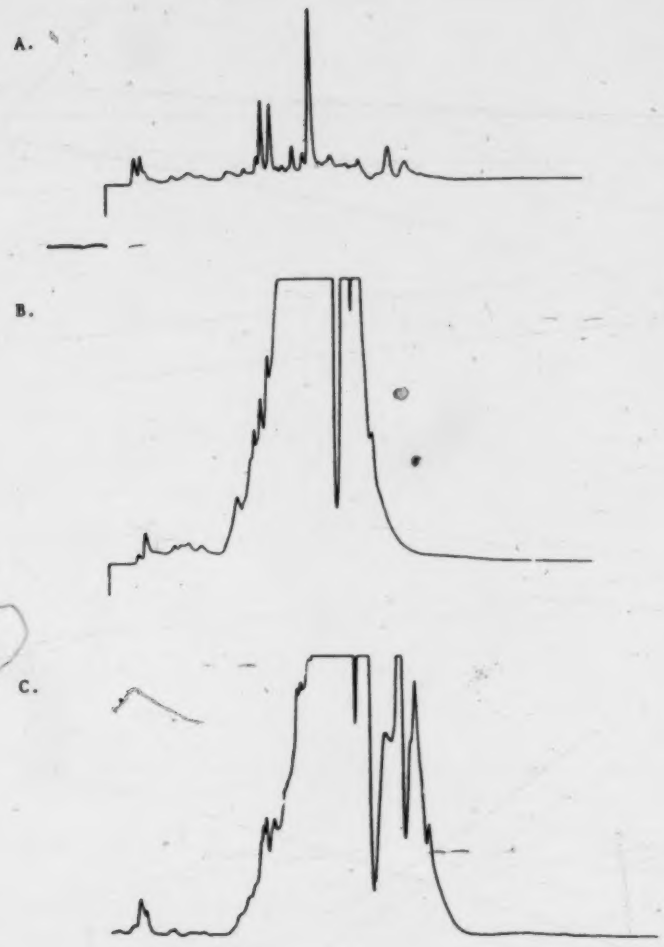


Figure 17. HPLC chromatograms of raw bile injections from: control-trout sampled at (oilsand-free) Winnipeg, Manitoba (upper); trout exposed to treated tailings pond water (middle); and trout exposed to Mildred Lake water only (lower).

oilsand-free Winnipeg, Manitoba revealed comparatively little fluorescence.

Bile from trout exposed to Mildred Lake water only exhibited a greater fluorescence response than bile from the most-tainting exposure water, namely treated tailings pond water. This result is unexpected. Because the Mildred Lake intake is downstream from the Suncor discharge, it is possible that the Mildred Lake response represents an extremely sensitive response to very low levels of hydrocarbon exposure. As well, the Athabasca River flows through some natural outcrops of bitumen which may contribute low levels of hydrocarbons to the river and thereby to Mildred Lake. However, no firm conclusions can be made from the results of this investigation concerning the relationship of fish tainting and HPLC fluorescence response.

5.3.2 Enzymatic hydrolysis of bile. Based on the GC-FID peak areas given in Figure 18, a recovery of 71% of a 1-naphthyl glucuronide spike was calculated.

Table 12 provides the concentrations of phenols in bile from fish exposed to treated tailings pond water. These results, based on GC-FID chromatogram peak areas (Figure 19), are supported by GC-MS TIC's of an alkylated

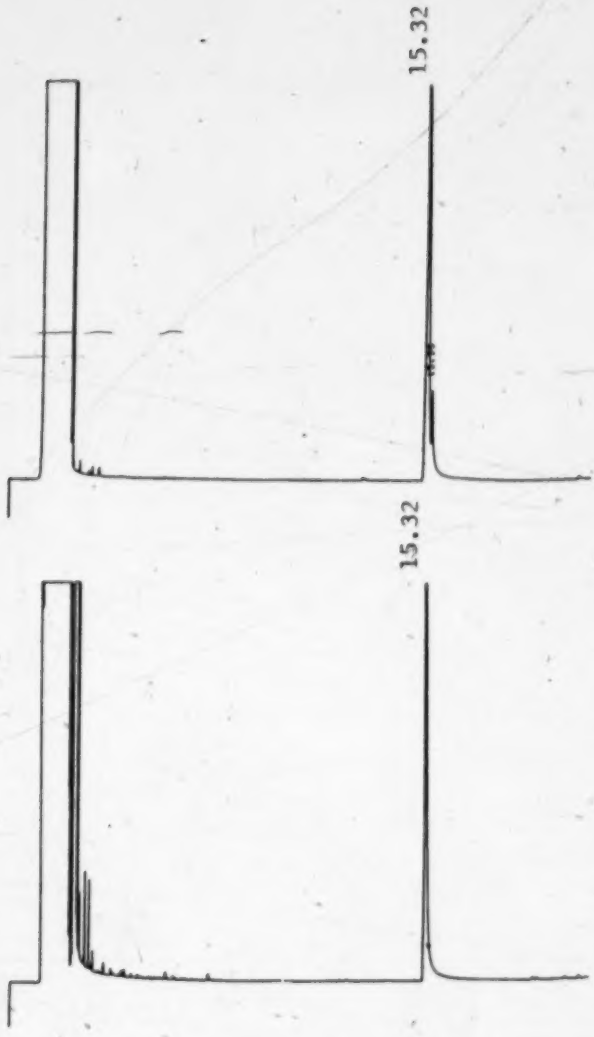


Figure 18. GC-FID chromatograms of a 1-naphthol standard (upper); and fish bile to which 1-naphthyl glucuronide had been added, and then enzymatically hydrolyzed (lower).

Table 12. Alkylated phenols (µg/g) in bile from fish exposed to treated tailings pond water (TTP); analysis in triplicate[a].

	mean ± standard deviation
	TREATED TAILINGS POND EXPOSED FISH BILE
phenol	0.3 ± 0.6
2-cresol	17.4 ± 3.6
3 and/or 4-cresol	19.2 ± 2.9
2,3 and/or 3,5-dimethyl phenol	17.5 ± 1.4
2,4 and/or 2,5-dimethyl phenol	88.4 ±20.5
2,6-dimethyl phenol	0.6 ± 1.0
3,4-dimethyl phenol	10.7 ± 0.6
Total phenol:	0.3
Total cresols:	36.6
Total dimethyl phenols:	117.2
Total phenol, cresols and dimethyl phenols	154.1

[a]Individual results are presented in Table 18, Appendix I.

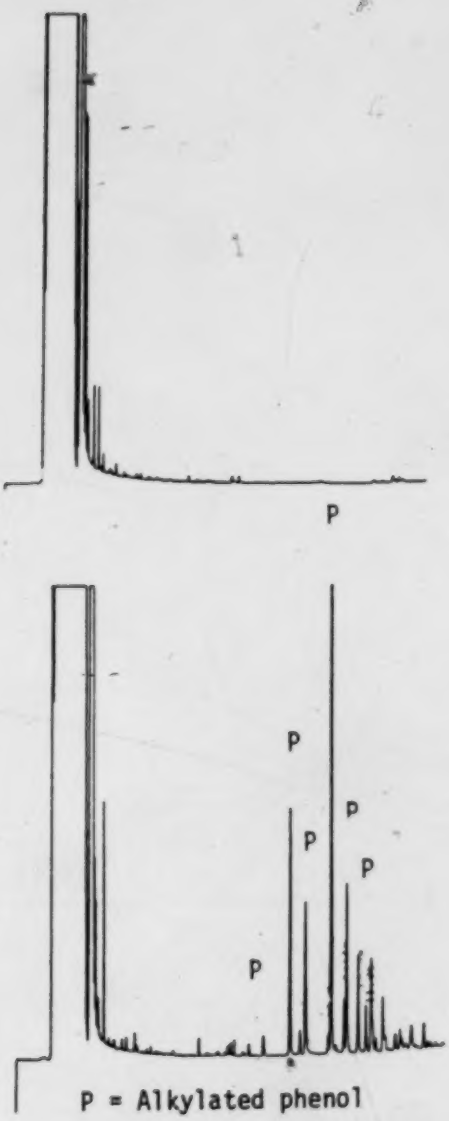


Figure 19. GC-FID chromatograms of hydrolyzed bile from trout held in Mildred Lake water only (upper), and in treated tailings pond water (lower).

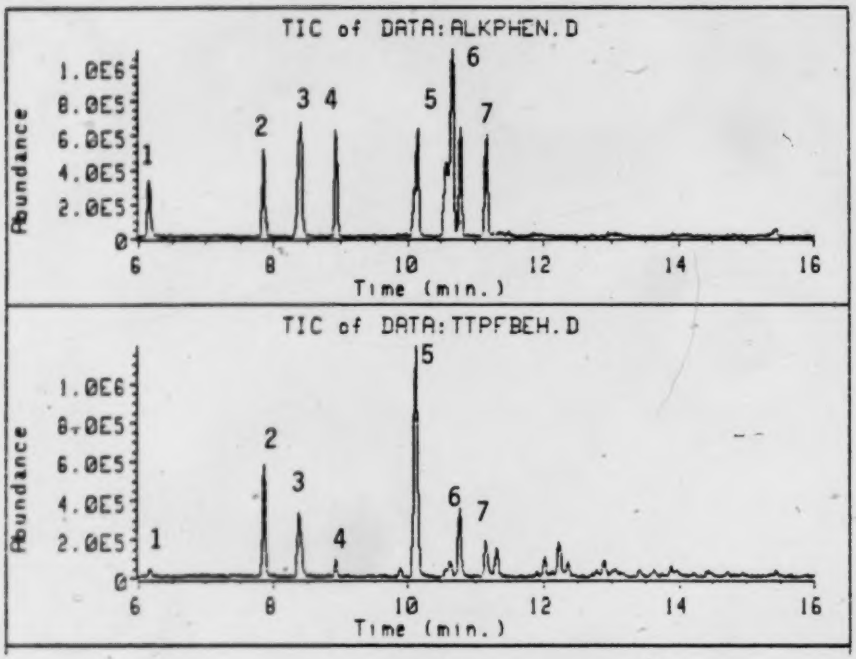
phenol standard and the TTP bile extract given in Figure 20.

Whereas phenol concentrations in TTP water and TTP water exposed fish fillet were in the ppb range, in TTP bile - phenols were recorded in the ppm range.

Apart from treated tailings pond water, and six percent whole tailings pond water (Figure 21), no other water exposure produced any detectable phenols or any other metabolites in the enzymatically hydrolyzed bile extracts.

The effect of the glucuronidase enzyme on phenol metabolites in bile can be seen in Figure 22. Without this enzyme, no metabolites would be detected. Phenols, likely of tailings pond origin were conjugated with glucuronic acid to assist in their excretion. Another possibility is that the trout were taking up xylenes from the tailings pond water and oxidizing them to dimethyl phenols (xylenols), either before or after conjugation with glucuronic acid. In their conjugated form these compounds are not volatile enough to be detected by GC-FID.

Since no metabolites of any sort were found in the catchment basin and one-year stored tailings pond exposed bile extracts any attempt to form a relationship between bile metabolites and fish tainting are stymied.



Peak	Compound
1	phenol
2	2-cresol
3	3 and/or 4-cresol
4	2,6-dimethyl phenol
5	2,4 and/or 2,5-dimethyl phenol
6	2,3 and/or 3,5-dimethyl phenol
7	3,4-dimethyl phenol

Figure 20. GC-MS comparison of alkylated phenol peaks in a phenol standard (upper box), with those identified in hydrolized bile from fish exposed to treated tailings pond water (lower box).

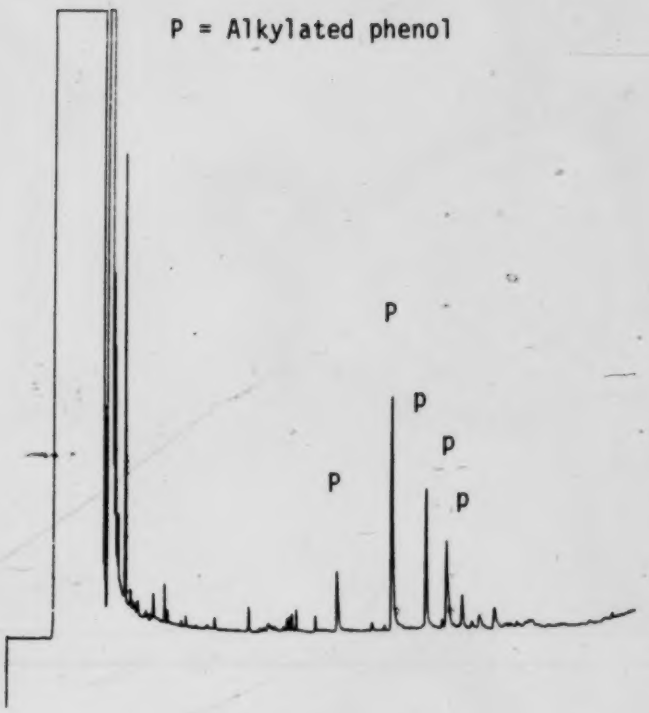


Figure 21. GC-FID chromatogram of bile extract from fish exposed to 6% whole tailings pond water.

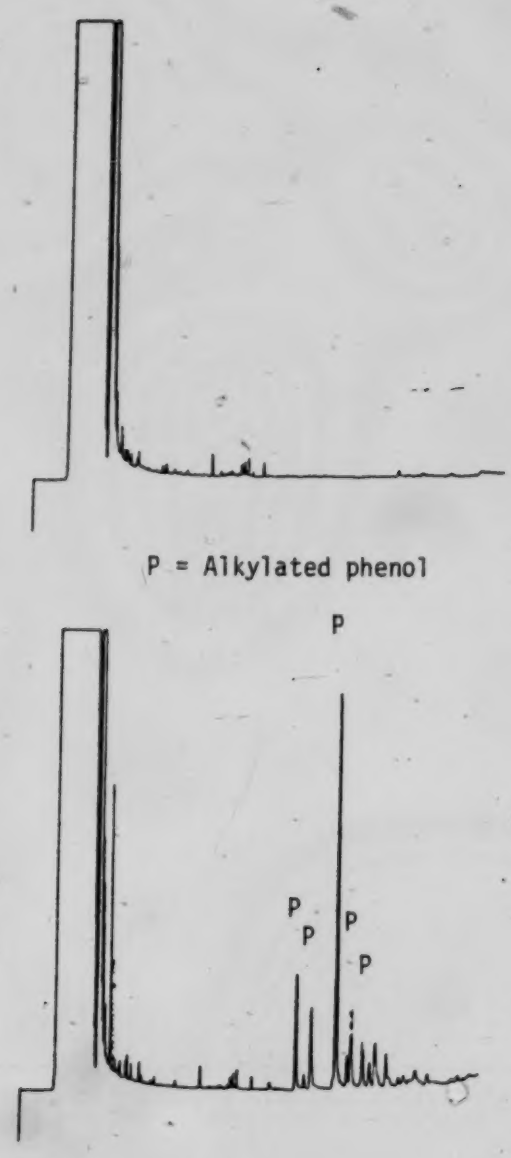


Figure 22. GC-FID chromatograms of bile from trout exposed to treated tailings pond water, revealing the zero effect of buffer (only) on bile hydrolysis (upper), and buffer plus enzyme (lower).

It may be that other (conjugates) types of metabolites are present, which do not respond to glucuronidase enzyme, eg. mercapturates.

Most fish bile analysis for petroleum compounds, either by HPLC or enzymatic hydrolysis has been performed on marine fish. Perhaps freshwater fish are markedly different with respect to their metabolism and excretion mechanisms of xenobiotic organic compounds.

Bile analysis was performed in triplicate in order to determine the reproducibility of the results, as was the sensory analysis. Because of time constraints there was no replication of chemical analyses of the wastewater and fish fillet tissue, therefore their reproducibility is unknown.

5.4 TISSUE ANALYSIS

Table 13 provides the percent recovery of alkylated naphthalenes, benzothiophene and dibenzothiophene which had been added to a fish fillet sample and then recovered in the florisil-hexane fraction. The average percent recovery was 81%. More volatile compounds such as the alkylated benzenes, phenols and thiophenes would likely have a lower recovery because of the many steps in this method during which they could be lost to the surrounding air.

Table 13. Percent recovery of selected aromatics which had been added to fish fillet tissue, florisil - hexane fraction.

Compound	Percent Recovery
naphthalene	79 %
benzothiophene	80
1-methyl naphthalene	78
2,6-dimethyl naphthalene	76
2,3,5-trimethyl naphthalene	74
dibenzothiophene	99

From Table 14 it is apparent that phenols will not elute in the florisil hexane (non-polar solvent) fraction. DCM, a polar solvent, combined with a 10% (distilled water) deactivation of florisil was required to obtain full recovery of an alkylated phenol spike. All alkylated benzenes, PAH's, and PASH compounds, being less polar, are eluted in the less polar hexane fraction.

Alkylated benzenes were identified by GC-FID and GC-MS in the hexane fractions of some of the trout fillet tissues (Figure 23), however it was later found that some of the hexane solvent used in the GPC and florisil cleanup procedures may have been contaminated with alkylated benzenes. Therefore no alkylated benzene concentrations in tissues can be reliably deduced from the results.

Table 15 provides the concentrations of alkylated phenols found in fillet tissue from fish exposed to treated tailings pond water, based on GC-FID peak areas from the florisil cleanup extract, DCM fraction. These GC-FID identifications are supported by the GC-MS results illustrated in Figure 24, where the tissue extract TIC is compared to a TIC of an alkylated phenol standard. Figure 25, showing mass spectra of dimethyl phenols in TTP water exposed fillet tissue, provides further support.

A comparison of GC-FID chromatograms of TTP and MLK water exposed fillet tissue (Figure 26) reveals the

Table 14 Percent recovery of alkylated phenols spiked onto deactivated florisil columns.

	% recovery from either 5 or 10% deactivated floricil - Hexane fraction	% recovery from 5% deactivated florisil - DCM fraction	% recovery from 10% deactivated florisil - DCM fraction
Phenol	0	72	103
2-cresol	0	73	102
3 and 4-cresol	0	35	54
2,3 and 3,5 dimethyl phenol	0	71	101
2,4 and 2,5 dimethyl phenol	0	72	100
2,6 dimethyl phenol	0	73	100
3,4 dimethyl phenol	0	69	101

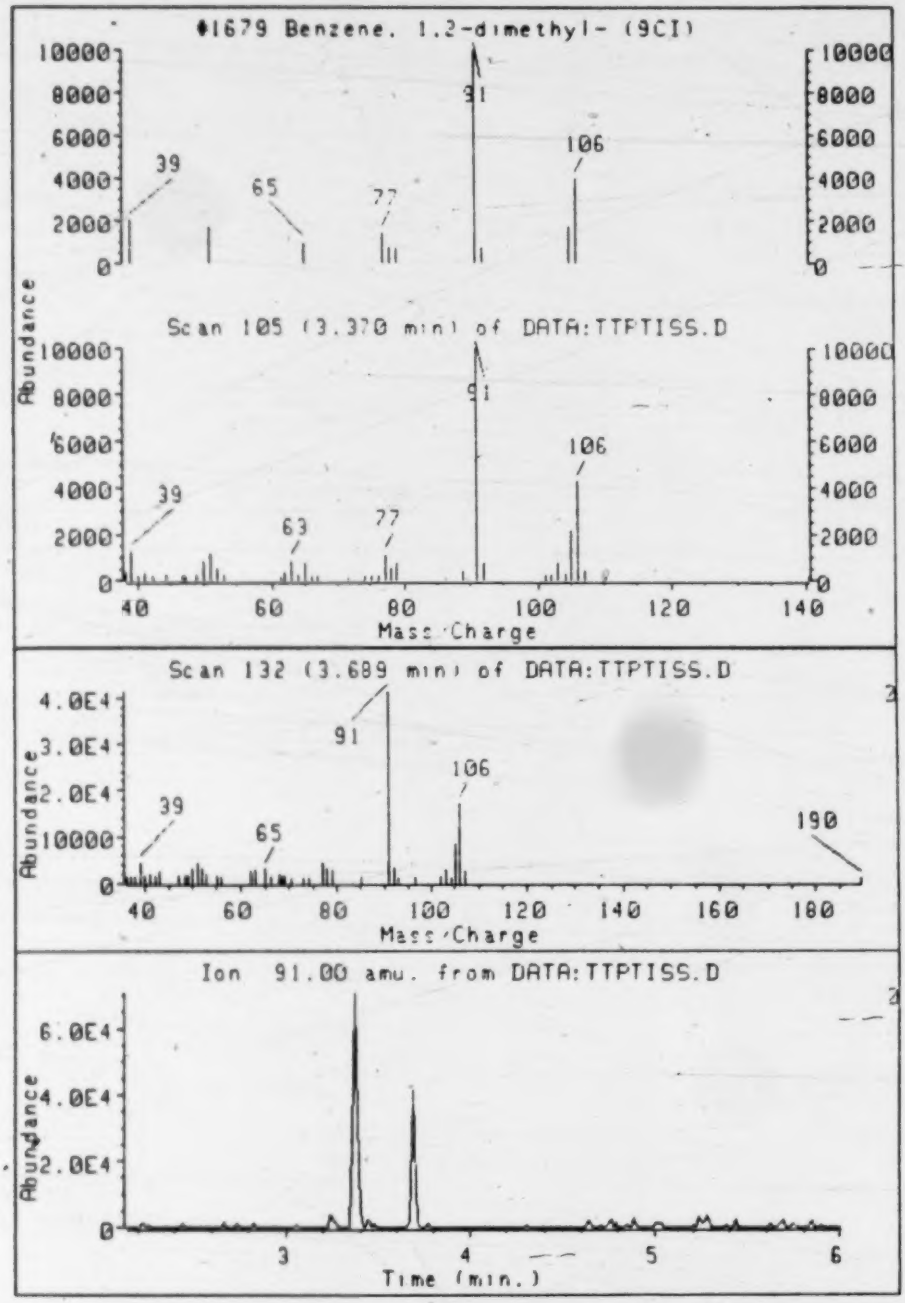
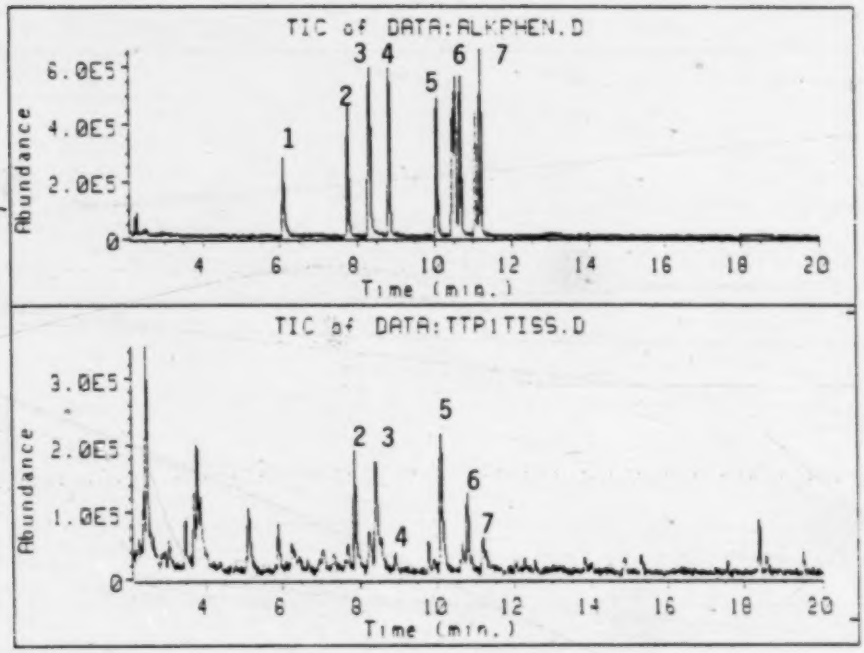


Figure 23. Xylenes as revealed by GC-MS in fillet tissue from fish exposed to treated tailings pond water, florisil - hexane fraction, possibly due only to contaminated hexane.

Table 15. Alkylated phenols in treated tailings pond (TTP) exposed fish fillet.

	Average of two fish (µg/Kg)[a]
phenol	46.3
2-cresol	125.0
3 and/or 4-cresol	135.0
2,3 and 3,5-dimethyl phenol	108.8
2,4 and 2,5-dimethyl phenol	165.4
2,6-dimethyl phenol	13.8
3,4-dimethyl phenol	67.6
Total phenol:	46.3
Total cresols:	260.0
Total dimethyl phenols:	355.6
Total phenol, cresols and dimethyl phenols:	661.9

[a]Calculated from GC peak areas.



Peak	Compound
1	phenol
2	2-cresol
3	3 and/or 4-cresol
4	2,6-dimethyl phenol
5	2,4 and/or 2,5-dimethyl phenol
6	2,3 and/or 3,5-dimethyl phenol
7	3,4-dimethyl phenol

Figure 24. GC-MS TIC comparison between phenols in a standard (upper box) and phenols identified in fillet tissue of fish exposed to treated tailings pond water, florisil - DCM fraction (lower box).

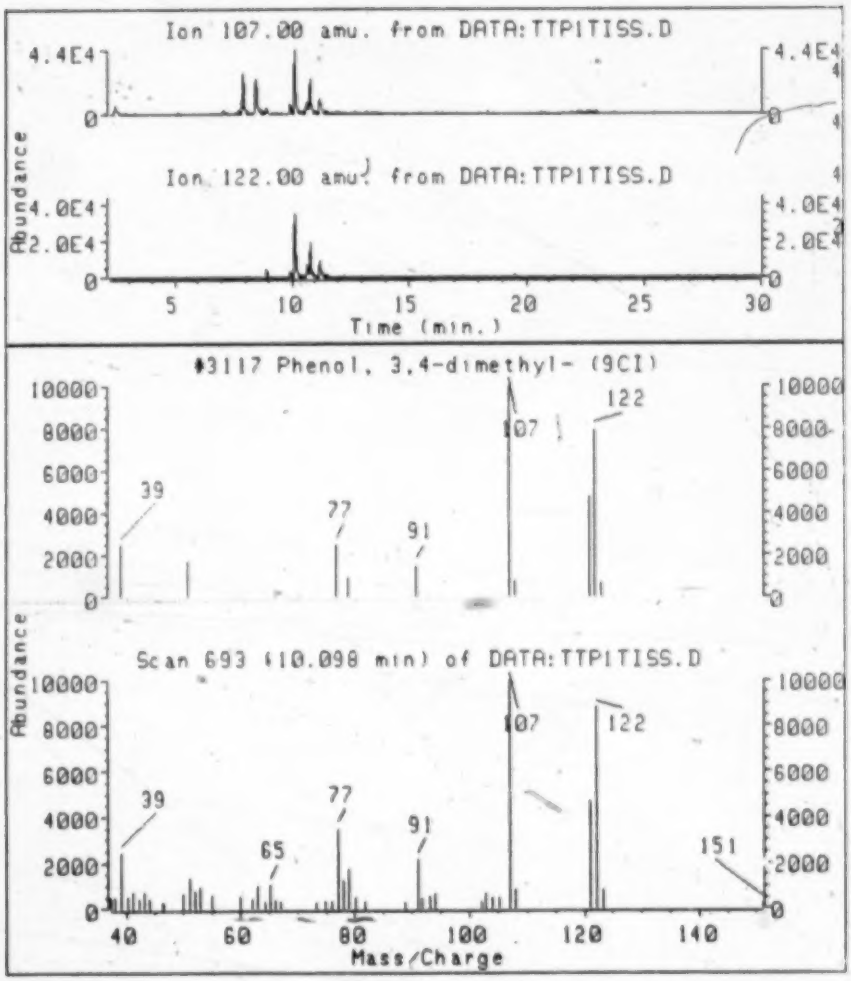


Figure 25. GC-MS selective ion detection of dimethyl phenols in fillet tissue of fish exposed to treated tailings pond water, florisil - DCM fraction.

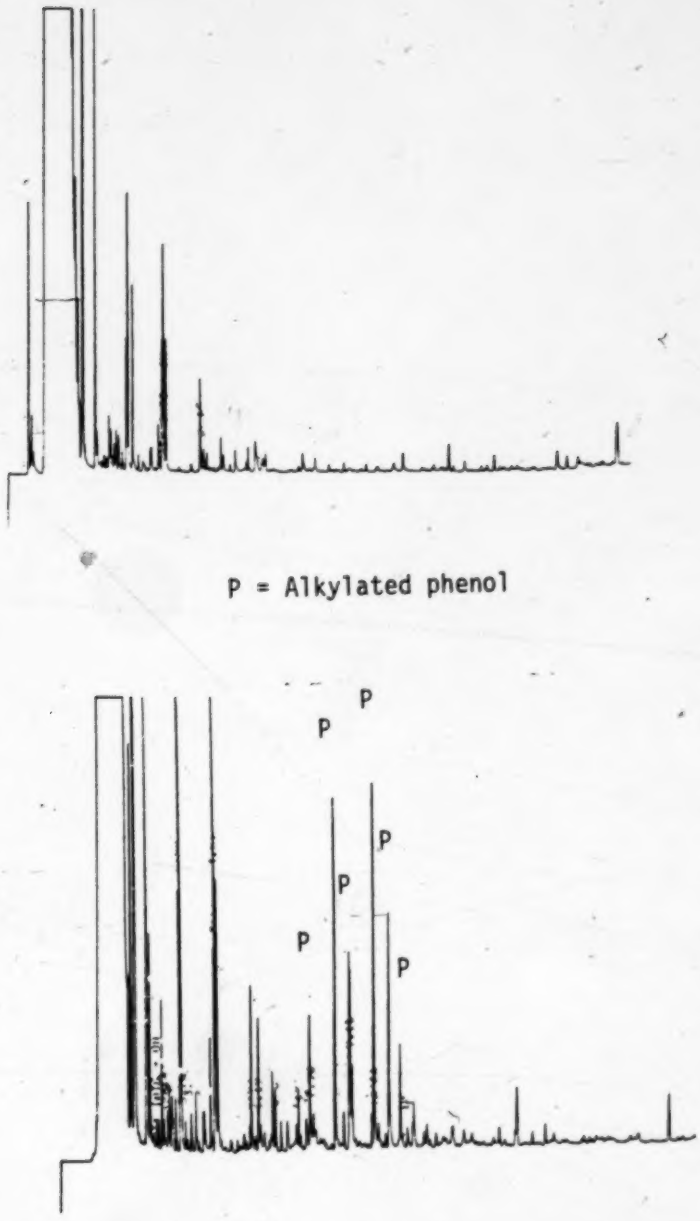


Figure 26. GC-FID chromatograms revealing akylated phenols in fillet tissue of fish exposed to treated tailings pond water (lower), and the absence of phenols in fillet from fish held in dechlorinated water at the University of Alberta (upper), florisil DCM fractions.

absence of phenols in the MLK water exposure. The GC-FID chromatograms are supported by GC-MS scans of the two major ion fragments of dimethyl phenols (ions 122 and 107) in TTP and MLK fillet extracts, as shown in Figure 27.

The most abundant alkylated phenol detected in the fillet tissue (as well as in the bile and wastewater) was 2,4 and/or 2,5 dimethyl phenol. The level of 2,4 and/or 2,5 dimethyl phenol detected (165 ppb) is near the threshold odor level reported by Jardine and Hrudey (1987) of 210 ppb (ng/g, or µg/kg). The total amount of all dimethyl phenols (356 ppb) and all phenols (662 ppb) are well above the reported sensory threshold. However, because of the presence of PASH and other petroleum compounds, and in addition the possibility of synergistic effects (mentioned in Section 3.3), it is unlikely that the phenols were solely responsible for the very strong taint detected with the fish exposed to treated tailing pond water.

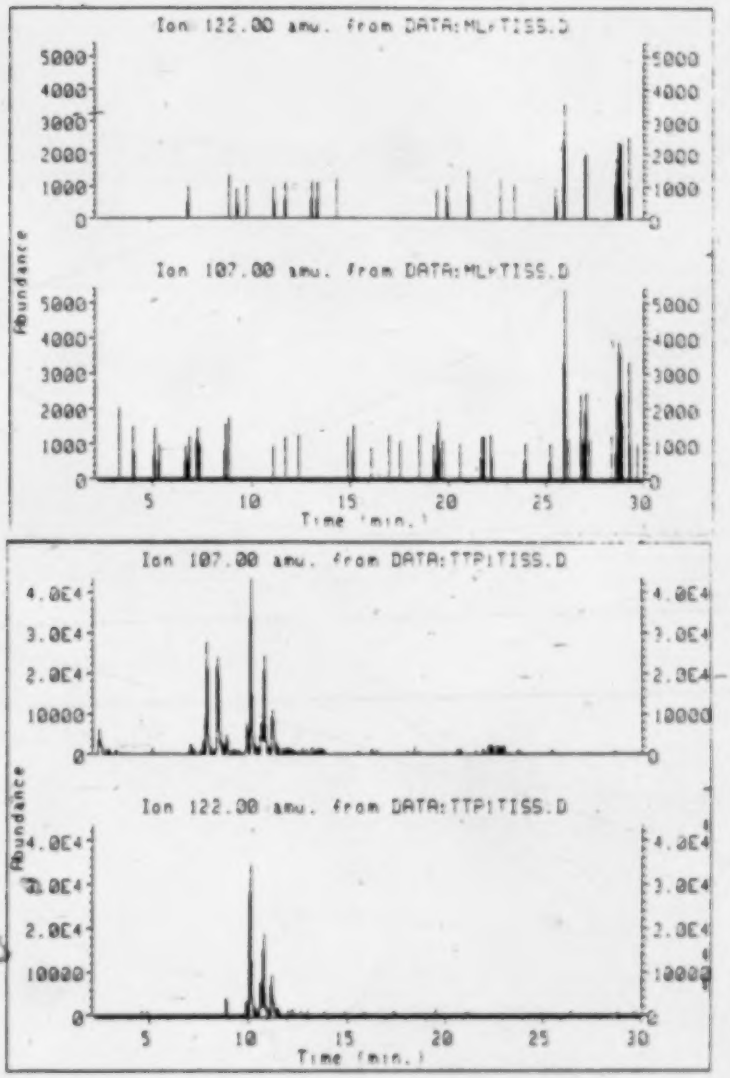


Figure 27. Comparison between abundance of ions 107 and 122 in fillet tissue of fish exposed to treated tailings pond water (lower box) and those exposed only to Mildred Lake water (upper box), Floricil - DCM fraction.

6. CONCLUSIONS

Sensory evaluation revealed that three of the four selected tailings pond wastewaters significantly tainted rainbow trout after 24 hours exposure. In the fourth case, unexpected toxicity problems encountered with the one year "stored" tailings pond water forced the fish that were still alive to be harvested after only 2.5 hours exposure. A significant taint was nevertheless recorded following this short exposure period.

Treated tailings pond water caused the most detectable taint of the four tailings pond derived wastewaters tested. The presence of phenol, cresols and dimethyl phenols were tentatively identified by GC-FID and confirmed by GC-MS in treated tailings pond (TTP) wastewater, and in fillet tissue and bile from rainbow trout exposed to TTP water. These compounds were not present in Mildred Lake reference water, fish fillet and bile samples.

Whereas concentrations of dimethyl phenols in TTP water and TTP water exposed fish tissue were quite similar, TTP water exposed fish bile showed much higher concentrations of dimethyl phenols.

Total concentration of dimethyl phenols in fillet tissue from fish exposed to treated tailings pond is above the detection threshold for 2,5-dimethyl phenol

reported by Jardine and Hrudey (1987). On this basis it can be concluded that phenols added to the taint of at least one of the selected tailings pond wastewater fish exposures, namely for the most-tainting wastewater - treated tailings pond water. However, because other petroleum compounds were present, and because of the potential effect of synergism, the total phenols concentration was likely insufficient to explain the strong taint observed with the fish exposed to treated tailings pond water.

Organic sulphur (PASH) compounds were identified by GC-FPD in tailings pond wastewaters. In fact, there was a correspondence between the concentration of organic sulphur compounds in the exposure waters, and the "detectablity of taint" from the sensory evaluation. Sulphur compounds however were not detected in bile or tissue from any of the exposed fish analyzed. Therefore no direct relationship between concentration of benzothiophene-like sulphur compounds and "detectablility of taint" was demonstrated.

Bile analysis by HPLC may provide a relatively simple and inexpensive method for monitoring the presence of oil compounds in lakes and rivers. However, the finding of high levels of bile fluorescence in untainted Mildred Lake reference fish indicates that more research

is needed to understand what compounds are contributing to the observed fluorescence response.

7. RECOMMENDATIONS

Because the fish tainting potential of tailings pond wastewaters has been demonstrated, this impact should be an important consideration in controlling tailings pond discharges to the Athabasca River.

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APPENDIX I

1. Table 16. Data from the sensory analysis performed on fish exposed to tailings pond waters.

2. Table 17. Statistical table used to determine whether a significant difference in taint occurred between fish exposed to test waters, and fish exposed to Mildred Lake water only.

3. Table 18. Alkylated phenols (µg/g) in three samples of hydrolyzed bile from fish exposed to treated tailings pond water.

Table 16. Data from the sensory analysis performed on fish exposed to tailings pond waters.

JUDGES	SESSION	MLK	UA(1)	MLK	UA(2)	MLK	TAP	MLK	11 YR	MLK	6M	MLK	CB	MLK	TP
1	1		1	1		1			2		1		2		9
	2	1			1	2			3		1		6		9
	3		1		1		1		4		2		6		9
2	1	1		1			4	1			3	1			9
	2	1		2			3		1		5		1		9
	3	1		1			3		1		7		3		9
3	1	0			2	2			5	1			8		9
	2	4		3			1		9	2			9		9
	3	1		3			1		7		5		6		9
4	1	1		1		1			9		1	1			9
	2	1		2			2		9	1			9		9
	3	1		1			5		9		4		8		9
5	1		1		4		2	4			7		8		9
	2	2		5			6		6		5		8		9
	3		4		1		3		8		6		7		9
6	1	5			3	3			8	2			6		9
	2	1		2			1		5		2		5		9
	3	3			3	1			6		7		6		9
7	1		1	1			1		7		1		6		5
	2		2	1			6		7		3		5		8
	3	4		2		4			9		3		1		7
8	1		1	4			2	3			5		9		6
	2		1	3		1			4		2		2		9
	3	2		3			2		5	2			8		9
9	1		3	5		7			9	5			2		9
	2				3		5		8		6		8		9
	3	6			7		7		9		7	7			9
10	1		6	3		2		1			9	1			9
	2		1		3		4		7		7		5		9
	3	3		4		3			5		7		3		9

This table provides a list of judges in column one, sessions in column two, and the judgements given in the "paired comparision" test in the remaining columns. The numbers (1-9) provide the "detectability of taint" noted by each judge (panelist), with "9" being the maximum detectable taint.

NOTE:

The material originally displayed on this page has been removed due to the unavailability of copyright permission.

The material removed contained a one-tailed and two-tailed statistical table that was used to determine whether a significant taint occurred between fish exposed to test waters, and fish exposed to the Mildred Lake reference water only.

The original source of this material is:

Roessler, E.B., G.A. Baker, and M.A. Amerine. 1956. One-tailed and two-tailed tests in organoleptic comparisions. Food Research. 21:117-121.

Table 18. Alkylated phenols (µg/g) in three samples of hydrolyzed bile from fish exposed to treated tailings pond water.

Compound	Bile sample number 1	2	3
phenol	0.	1.0	0.
2-cresol	13.9	21.0	17.4
2,6 dimethyl phenol	0.	1.8	0.
3 and 4-cresol	16.6	22.3	18.6
2,4 and 2,5 dimethyl phenol	111.0	83.3	70.9
2,3 and 3,5 dimethyl phenol	16.1	18.8	17.5
3,4 dimethyl phenol	10.4	11.4	10.4